THIRD REPORT ON A TEST OF McDOUGALL'S LAMARCKIAN EXPERIMENT ON THE TRAINING OF RATS

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(With Two Text-figures)

Our First Report on this experiment was published in 1935, and dealt with the first five generations. The Second Report, 1942, included the first twenty generations. This Third Report adds another sixteen generations, and reviews the whole experiment up to date.

The nature of the experiment is well known. The rats are 'trained' (but actually have to discover for themselves) to choose the less brightly illuminated of two exits from a tank full of water, and the number of errors (choices of the bright exit) made before they take to using the dim exit exclusively, is recorded generation by generation. McDougall's experiment extended over forty-four generations, but records of only the last thirty-two are available and relevant. Over these thirty-two generations McDougall records a marked and progressive (though of course not quite regular) decrease in the number of errors made in successive generations. Unfortunately, he made the irremediable mistake of failing to maintain a parallel control line for comparison with his trained line. Crew repeated the experiment, with some inessential modifications of training technique, and found no evidence of a decrease in the number of errors during the eighteen generations of his experiment.

As it is five years since we published our last report, we will repeat a brief description of the methods employed. Further details are to be found in our First Report.

The apparatus is essentially as figured by McDougall (1930). It consists of a tank, containing water, divided into three parallel passages by two partitions which stop short of the far, curved end of the tank. At that end, therefore, the passages communicate. From the near end of each side passage a gangway in the form of a wire ladder leads to a platform above the water-level. The rat is placed in the water at the near end of the central passage. In its search for a way to escape from the water it swims down the central passage, turns right or left into one of the side passages, and swims back along this to the gangway at the end of it and climbs out. Behind a sheet of ground glass at the back of each gangway is a low-power electric lamp, which shines through it down the whole length of the passage, illuminating also its communication with the central passage, so that the rat at its starting-point in the central passage can see which of the two side passages is illuminated. The rat is

given six trials a day (except for the first 5 days of training, when, unlike McDougall, we give only four trials) with the left and right passages illuminated alternately. The gangways are connected with an alternating current in such a way that the current is thrown into whichever gangway is illuminated, but not into the other. The rat can therefore escape from the tank by either gangway, but if it chooses the illuminated ('bright') one it does so at the expense of a 3 sec. electric shock. The rat has to learn always to escape by the dim gangway, irrespective of whether this is on the right or left. Facility in learning the task is measured by the number of errors, that is to say, number of escapes by the bright gangway, made by the rat before it learns to avoid this and always chooses the dim one. Occasionally a rat makes contact with the electrified gangway, but instead of climbing it, turns back on receiving the shock, swims to the other exit and escapes by it. This very rarely happens except during the first few days of training. This is recorded as an error. The number of errors made is therefore the same as the number of shocks received. A rat is held to have learnt the task as soon as it has made twelve consecutive correct runs. Further details of the apparatus and training procedure are set out in our First Report, and have not been changed since. The various factors which influence the rate of learning are also discussed in that report, especially those which appear to be responsible for the great variation in the number of errors made by individual rats even of the same litter.

All the rats are descended from a single pair of albino rats of Wistar origin. The first generation obtained from this pair (which was not trained) was divided into two groups, one of which (five rats) was trained and became the ancestors of the trained line; the other group (four rats) was not trained and became the ancestors of the control line. The two lines have been bred parallel with each other, and under the same conditions. In each generation the required number of rats in the trained line are trained, and mated as parents of the next generation. In the control line some of the litters produced are not trained, but are kept as parents of the next generation. Other litters of this line are trained to provide controls to the same generation of the trained line. These trained controls are, of course, not used for breeding. In this way each generation of the trained line is tested against an approximately equal number of trained controls, differing from the trained line only in the fact that their ancestors were not trained.

At the time of weaning each rat is given its individual mark by means of dye marks on different parts of the body, a different colour being used for each of the three groups, the trained line, untrained controls for breeding, and trained controls. These dye marks are renewed from time to time before they fade, so that track is kept of every rat throughout its lifetime. As an additional safeguard the three groups are permanently distinguished by ear-clippings.

Our system of mating ensures that the number of errors made by any rat in the trained line does not influence its chance of becoming a parent. The rats are weaned and given their identification marks when 26 days old; on the next day they are given six preliminary runs in the tank without the differential illumination of the gangways, or the shock. Training proper begins on the next day when they are

28 days old. As described in our First Report, the few rats which have not learnt after 52 days of training (by which time they are 80 days old) are given 'special training'. These slow learners are usually rats which, early in training, have formed the habit of using exclusively either the right- or left-hand exit passage, whether this is illuminated or not. Consequently after adopting this habit, they receive a shock on every alternate run. As the rats are given four trials a day for the first 5 days, and six a day thereafter, by the end of 52 days they have had 302 trials, and have received approximately 151 shocks. 'Special training' consists in forcing them for a few times to use the unfamiliar exit. After this they invariably learn to use the dim passage, either at once or after a very few days. Thus even the slowest learners have completed their task before the age at which any rat is mated. In fact, the reason for giving 'special training' to these rats is to ensure that slowness in learning shall not result in later age of mating (for they cannot be mated before they have learnt) and therefore in diminishing their chance of becoming parents of the next generation. The number of rats which receive this 'special training' can be seen from Table 2, where they are shown by the letter S.

The rats are mated without any reference to their training scores; we have avoided brother-sister matings as far as possible, except during a short period of the experiment to be referred to later. Not all the rats mated become parents of the next generation, for many of the matings prove infertile, and others do not produce litters till after the number of young required has been obtained. This, of course, applies to both the trained and control lines. It is our practice to reduce litters of more than six young to that number within a few days of birth, occasionally leaving as many as eight in second litters of large mothers.

Of the total 2848 rats which have started training, twenty-one died before achieving the criterion of learning to escape by the dim gangway. These twenty-one rats are excluded from our figures. Throughout the whole of this experiment there have been no deaths, or injuries of any sort, attributable to the electric shock.

MEASURE OF CENTRAL TENDENCY OF TRAINING SCORES

In both our previous reports we have discussed the problem of finding a satisfactory measure of the performance of a group of rats as a whole. McDougall's method of measuring this by the arithmetical mean number of errors made by the rats is unsatisfactory owing to the extreme skewness of the distribution (as shown by our Second Report, Table 1). Moreover, such a measure is further invalidated in our case by our practice of giving 'special training' to rats which have not learnt after 52 days of training. In our Second Report we adopted three measures, the median score, and the percentages of rats learning with less than ten and more than 100 errors. We have now reverted to the type of measure we used in our First Report, but based on much larger numbers. The scores of the first thousand control rats were arranged in order of magnitude, and the whole group divided into ten classes, each containing as nearly as possible an equal number of rats, having regard to the fact that the number of errors are necessarily whole numbers. The resulting distribution is shown in Table 1.

Thus all rats with training scores 0–5 errors inclusive are placed in class 1, and so on. The arithmetic mean of the classes so obtained will be referred to as the *mean class* of the group of rats concerned.

Table 1. *The first* 1000 *controls classified according to the number of errors made*

Class	1	2	3	4	5	6	7	8	9	10
No. of errors	0–5	6–10	11–15	16–19	20–23	24–27	28–32	33–40	41–67	68+
No. of rats	93	110	102	105	93	96	106	95	99	101

THE PERFORMANCE OF THE RATS IN THE TRAINED AND CONTROL LINES (LINES T AND C)

Table 2, which is a continuation of Table 2, Second Report,* with the addition of the column *Mean class*, gives the training scores of every rat since our last report. Table 3 gives the figures for the whole experiment to date, in groups of four generations. As, however, no rats were trained in the first generation of controls, the first group contains only generations 2, 3 and 4 of line C. The mean class and percentages are the weighted figures, i.e. each group of four generations is taken as a single unit.

In our Second Report we drew attention to periodic changes in facility of learning (as measured by the number of errors made) which ran roughly parallel in the trained and control lines. That this tendency has continued is shown by Table 3, and in graphic form by Figs. 1 and 2. The greater smoothness of the graph in Fig. 2 is due to the large number of scores on which each point is based. For example, the violent fluctuation of line T in 1936 (Fig. 1) is based on only twenty-five rats.

It will be seen that during the first fifteen or sixteen generations, trained in 1932–9, there was a progressive decrease in the number of errors in both lines. Then, in spite of considerable fluctuations, both lines remained at this low level of errors, or even with a slight further decline till about generations 28–30, trained in 1944–5. About this time the number of errors began to rise again in both lines, this trend being continued to the end of the period under review (April 1947).

A glance at Table 3, column *Mean class*, and at Figs. 1 and 2, shows how the validity of McDougall's conclusions suffers from his failure to maintain a control line. Had we not done so, and if our experiment had stopped at about generation 28 —or even at generation 32 or 33—our results could have been used as evidence of the operation of a Lamarckian factor. But the parallel behaviour of the two lines throughout, as well as the later rise in the number of errors, excludes this interpretation.

We have no explanation to offer for these long-scale changes. As they are exhibited in a parallel way by both lines, between which there has of course been no cross-breeding since they were established from the same pair of parents in 1932,

* We take this opportunity of correcting three errors in this table. In generation 16 T, one S should be in heavy type, and in 16 C there should be another rat with score 25, and in 18 C another with score 33, giving the correctly stated total of fifty rats in each case.

Table 2. *Showing the number of errors made (shocks received) by each rat, the median number of errors, and the mean class, in each of generations 21–36*

Generation		No. of rats	Median	Mean class	Number of errors made by each rat
21	T	50	23·5	5·50	0, 2, 2, 2, 3, 3, 6, 7, 9, 10, 11, 11, 12, 15, 15, 17, 17, 17, 17, 18, 19, 19, 20, 21, 23, 24, 25, 25, 26, 27, 29, 31, 31, 31, 31, 35, 36, 38, 40, 41, 47, 55, 63, 80, 86, 89, 100, S, S, S
	C	34	11	3·29	0, 2, 2, 3, 4, 4, 5, 5, 5, 5, 6, 6, 6, 8, 8, 9, 11, 11, 12, 13, 14, 16, 16, 16, 17, 17, 22, 23, 26, 29, 30, 32, 36, 100
22	T	48	13·5	3·68	0, 0, 0, 0, 0, 0, 0, 0, 1, 1, 1, 2, 2, 2, 3, 4, 4, 4, 5, 7, 7, 7, 8, 12, 15, 15, 18, 18, 18, 19, 19, 20, 22, 22, 23, 23, 24, 25, 25, 28, 31, 31, 33, 59, 88, 103, 104, S
	C	50	23·5	5·34	0, 1, 1, 3, 3, 4, 5, 6, 6, 10, 11, 12, 13, 13, 14, 15, 15, 16, 16, 17, 19, 21, 22, 22, 23, 24, 24, 25, 26, 26, 28, 28, 29, 29, 30, 30, 33, 35, 37, 39, 39, 42, 43, 45, 49, 49, 75, 108, 109, S
23	T	49	16	4·08	0, 0, 2, 3, 3, 4, 4, 5, 5, 6, 7, 8, 8, 8, 9, 10, 10, 12, 13, 13, 14, 14, 15, 16, 16, 17, 18, 18, 18, 19, 19, 20, 20, 25, 25, 25, 26, 26, 27, 27, 28, 30, 33, 35, 36, 37, 40, 42, 90
	C	50	21·5	5·38	0, 3, 4, 6, 9, 10, 10, 10, 11, 12, 12, 12, 14, 14, 15, 15, 16, 19, 19, 19, 19, 19, 20, 21, 21, 22, 22, 24, 24, 25, 25, 28, 28, 30, 30, 31, 31, 34, 34, 35, 41, 42, 43, 44, 49, 50, 63, 99, 144, S
24	T	50	20	4·52	0, 2, 4, 6, 6, 9, 10, 12, 13, 13, 13, 13, 14, 15, 15, 17, 17, 17, 18, 18, 18, 18, 19, 19, 20, 20, 21, 21, 21, 22, 22, 23, 23, 23, 23, 23, 24, 24, 25, 25, 25, 28, 28, 29, 29, 29, 33, 35, 65
	C	49	18	4·51	0, 1, 2, 2, 2, 3, 3, 4, 5, 5, 6, 6, 7, 9, 11, 15, 16, 16, 17, 18, 18, 18, 18, 18, 18, 19, 19, 19, 19, 20, 21, 21, 22, 25, 25, 25, 27, 28, 28, 31, 32, 34, 37, 44, 47, 49, 141, 145, 152
25	T	50	12·5	3·46	0, 0, 0, 1, 1, 1, 1, 2, 3, 4, 4, 4, 4, 4, 4, 5, 5, 6, 7, 8, 10, 10, 11, 12, 12, 13, 13, 14, 14, 15, 18, 18, 18, 19, 19, 19, 21, 21, 21, 23, 24, 26, 28, 30, 31, 33, 33, 54, S, S
	C	50	20	5·00	2, 3, 3, 6, 7, 9, 9, 9, 10, 10, 10, 13, 13, 13, 13, 14, 14, 16, 16, 17, 18, 19, 19, 20, 20, 20, 21, 22, 23, 24, 24, 24, 24, 27, 28, 28, 30, 30, 31, 31, 33, 33, 36, 37, 40, 45, 46, 53, 75, 140
26	T	50	19·5	4·34	1, 2, 2, 2, 4, 4, 5, 5, 5, 5, 7, 7, 8, 8, 9, 10, 10, 12, 13, 13, 13, 15, 16, 17, 19, 20, 20, 20, 21, 21, 23, 23, 24, 25, 25, 25, 27, 27, 27, 27, 29, 31, 32, 34, 34, 36, 41, 66, 111, S
	C	50	15	4·54	4, 5, 5, 6, 6, 6, 6, 7, 7, 7, 7, 8, 8, 9, 9, 10, 10, 10, 11, 12, 12, 13, 13, 14, 14, 16, 16, 17, 20, 20, 21, 22, 22, 26, 27, 27, 28, 28, 29, 31, 32, 39, 41, 45, 53, 56, 67, 78, 121, S
27	T	39	16	4·15	1, 2, 3, 3, 4, 4, 5, 5, 5, 5, 6, 6, 6, 8, 9, 12, 13, 13, 15, 16, 16, 18, 19, 19, 20, 20, 22, 23, 27, 28, 32, 33, 34, 36, 39, 44, 48, 114, S
	C	50	15·5	4·10	3, 4, 4, 4, 4, 5, 5, 6, 6, 6, 7, 8, 8, 9, 9, 10, 12, 14, 14, 15, 15, 15, 15, 15, 15, 16, 16, 16, 17, 17, 19, 19, 20, 20, 21, 21, 22, 22, 23, 26, 26, 28, 37, 49, 50, 54, 87, 108, S
28	T	50	19	4·48	0, 0, 1, 1, 1, 1, 2, 3, 3, 4, 5, 6, 7, 7, 10, 10, 11, 12, 12, 14, 14, 15, 15, 18, 19, 19, 20, 21, 22, 22, 23, 23, 26, 26, 26, 27, 28, 30, 30, 32, 35, 36, 37, 40, 42, 42, 51, 52, 111, 140
	C	47	20	5·04	4, 5, 6, 6, 6, 7, 8, 8, 8, 8, 9, 10, 10, 11, 11, 12, 14, 14, 16, 16, 17, 18, 19, 20, 21, 22, 24, 25, 25, 26, 27, 27, 28, 28, 30, 33, 34, 36, 39, 39, 41, 41, 43, 53, 79, 101, S
29	T	49	22	5·39	0, 1, 4, 5, 5, 6, 7, 13, 13, 13, 13, 14, 16, 16, 17, 18, 20, 20, 20, 21, 21, 21, 22, 22, 22, 23, 24, 24, 24, 25, 25, 26, 26, 27, 28, 32, 35, 36, 37, 37, 38, 42, 44, 45, 46, 47, 50, 50, 52
	C	50	21·5	5·08	1, 1, 4, 4, 5, 5, 6, 7, 10, 10, 10, 11, 12, 12, 13, 13, 13, 13, 15, 15, 16, 17, 19, 20, 21, 22, 26, 26, 26, 26, 26, 27, 27, 28, 29, 29, 30, 30, 30, 33, 38, 40, 41, 41, 45, 45, 77, 141, S, S
30	T	25	12	3·88	0, 1, 3, 3, 3, 3, 5, 8, 8, 9, 9, 9, 12, 13, 15, 18, 21, 22, 25, 25, 28, 37, 75, S, S
	C	24	22	5·54	5, 9, 9, 10, 10, 13, 14, 16, 16, 19, 21, 22, 22, 23, 27, 28, 30, 37, 51, 66, 93, 102, 103, 135
31	T	36	20·5	4·89	4, 5, 5, 5, 6, 6, 8, 9, 12, 15, 15, 16, 16, 17, 17, 17, 19, 20, 21, 21, 22, 23, 25, 25, 27, 30, 30, 31, 33, 35, 36, 36, 37, 39, 41, 151
	C	26	24	5·50	4, 5, 5, 6, 8, 9, 12, 14, 17, 20, 21, 22, 23, 25, 26, 28, 28, 31, 33, 35, 37, 42, 44, 66, 76, S
32	T	50	21·5	5·26	2, 4, 4, 6, 7, 7, 9, 12, 13, 13, 13, 14, 14, 15, 15, 15, 17, 18, 18, 18, 19, 20, 20, 21, 21, 22, 22, 22, 22, 23, 24, 24, 27, 28, 28, 28, 28, 28, 29, 36, 37, 41, 41, 46, 52, 54, 59, 89, S, S
	C	50	16·5	4·48	4, 4, 5, 6, 6, 7, 7, 7, 8, 9, 10, 11, 11, 11, 12, 12, 13, 13, 14, 14, 15, 15, 15, 16, 16, 17, 17, 18, 19, 20, 22, 22, 23, 23, 23, 25, 26, 27, 27, 28, 30, 31, 32, 33, 35, 41, 56, 60, S, S
33	T	31	26	5·71	1, 4, 8, 10, 13, 13, 13, 15, 18, 20, 20, 21, 25, 25, 25, 26, 26, 27, 27, 28, 29, 30, 33, 37, 38, 40, 41, 41, 57, 63, 64
	C	49	21	4·88	3, 4, 5, 6, 7, 7, 8, 8, 9, 9, 10, 11, 12, 13, 15, 15, 15, 15, 15, 16, 16, 17, 20, 20, 21, 21, 21, 22, 23, 23, 24, 25, 25, 25, 27, 27, 28, 31, 31, 31, 32, 34, 35, 41, 50, 54, 115, S, S
34	T	50	26	5·86	0, 1, 4, 5, 6, 6, 7, 9, 9, 11, 11, 12, 14, 15, 18, 19, 20, 20, 22, 23, 23, 24, 24, 25, 25, 27, 28, 28, 30, 30, 31, 31, 33, 35, 35, 36, 36, 36, 38, 40, 43, 47, 48, 51, 57, 71, 79, S, S, S
	C	50	32·5	7·12	5, 7, 12, 15, 16, 17, 17, 20, 20, 21, 21, 22, 23, 24, 24, 25, 25, 25, 26, 26, 29, 29, 30, 32, 32, 33, 33, 35, 35, 36, 38, 39, 41, 42, 46, 47, 47, 55, 56, 66, 92, 116, 122, 123, 124, 137, S, S, S, S
35	T	49	29	6·59	0, 6, 8, 12, 12, 17, 17, 18, 19, 21, 21, 23, 23, 24, 26, 26, 26, 26, 27, 27, 29, 29, 29, 29, 29, 30, 30, 30, 31, 33, 34, 35, 37, 37, 37, 39, 40, 40, 40, 42, 44, 46, 47, 56, 63, 75, 103, S
	C	50	27·5	6·72	7, 11, 13, 14, 17, 19, 19, 21, 21, 21, 23, 23, 23, 24, 24, 24, 25, 25, 25, 25, 25, 25, 26, 27, 27, 27, 28, 28, 28, 30, 30, 31, 31, 33, 33, 34, 36, 39, 40, 43, 45, 46, 47, 58, 62, 84, 116, S, S, S, S
36	T	50	29·5	6·58	8, 8, 11, 14, 15, 16, 17, 17, 19, 20, 20, 21, 21, 22, 22, 24, 24, 26, 26, 26, 27, 28, 28, 28, 29, 30, 30, 30, 31, 32, 32, 35, 35, 35, 37, 37, 37, 37, 39, 39, 39, 40, 42, 42, 43, 67, 147, S, S, S
	C	50	20·5	5·34	3, 4, 5, 6, 7, 8, 8, 9, 11, 12, 12, 13, 14, 14, 16, 17, 17, 17, 17, 18, 19, 19, 19, 20, 20, 21, 21, 21, 21, 22, 23, 24, 24, 25, 29, 30, 33, 37, 37, 41, 53, 57, 61, 61, 63, 73, 149, S, S, S

T, trained line; C, control line. S indicates that the rat failed to learn after 302 trials (and therefore with about 150 errors) and, if in the trained line, was given 'special training'. In the trained line the rats which became parents of the next generation are in heavy type.

Table 3. *Summary of the results of the thirty-six generations in groups of four generations*

Generations	No. of rats		Mean class			% in class 1		% in class 10	
	T	C	T	C	Ratio T/C	T	C	T	C
1–4	64	54	7·89	7·89	1·00	0·00	0·00	25·00	27·78
5–8	116	91	7·34	6·49	1·13	3·45	4·40	23·28	10·99
9–12	105	177	6·23	6·33	0·98	6·67	3·95	10·48	11·86
13–16	187	148	4·41	5·01	0·88	15·51	10·81	6·42	5·41
17–20	230	200	5·07	5·29	0·96	11·30	11·50	4·35	14·00
21–24	197	183	4·46	4·75	0·94	18·78	16·39	6·09	6·01
25–28	189	197	4·11	4·66	0·88	25·40	7·61	4·23	5·58
29–32	160	150	5·00	5·03	0·99	11·88	8·67	4·38	8·00
32–36	180	199	6·12	6·02	1·02	3·89	3·52	6·67	12·06

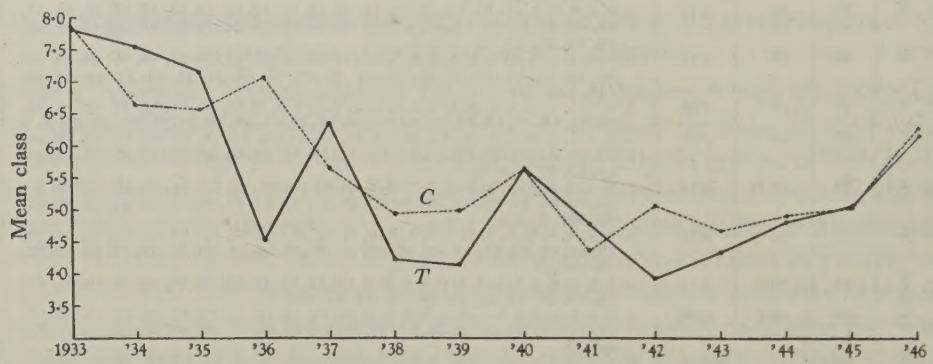

Fig. 1. Continuous line, Line T; broken line, Line C. The mean class are those of all rats which began training in the year referred to. The last point, 1946, includes most of generation 36 line T, but none of that generation in line C, which began training in January 1947.

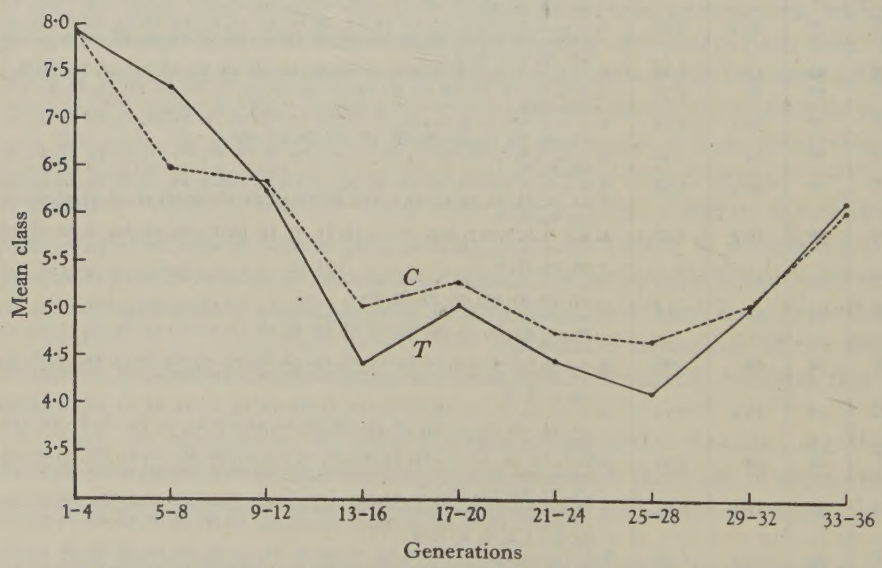

Fig. 2. This figure shows Table 3, column *Mean Class*, in form of a graph. Continuous line, Line T; broken line, Line C.

it is hardly possible that the factors concerned are genetic. Of possible environmental factors the one which immediately suggests itself is variation in the severity of the punishment for taking the bright gangway, which as McDougall showed (1930) influences the number of errors made. This must depend on the strength and duration of the electric shock. Our methods for keeping these constant are described in our First Report (p. 194); they have not been varied since. During the training of generation 35 the electrical resistance which had been in use from the beginning of the experiment was tested and found not to have altered to a perceptible degree, and the circuit between the resistance, the gangways and the water in the tank, which is closed by the rat placing its forepaws on the wire ladder with its body in the water, was found to be in perfect order. Moreover, in spite of their increasing persistence in taking the bright gangway during the last few generations, the rats' reaction to the shock on the electrified gangway is as vigorous as ever. The lamps used for illuminating the gangways are changed every 6 months as a matter of routine. We therefore have no reason to believe that the slow changes in rate of learning observed during the course of the experiment can be attributed to changes in training technique or apparatus. The diet and general husbandry has not been significantly varied since generation 17 (Second Report, p. 163). In fact, we have no explanation of these changes to offer. The fact that the fluctuations run parallel in the two lines excludes, however, not only the Lamarckian factor, but genetic factors in general.

There is, however, another fact brought out by Table 3 and Figs. 1 and 2. Although both lines participated in the decline in the number of errors during the first sixteen generations, line T reached a lower level than line C, and maintained its superiority until about generation 32, after which the mean classes of the four-generation groups are practically equal. The superiority of line T over line C during the sixteen or twenty generations was mainly, though not entirely, due to the excess of very quick learners in line T (Table 3, percentage in class 1; the violent fluctuations in the percentage are partly explicable by the comparatively small number of rats concerned).

It seems impossible to account for this prolonged difference between the two lines except by a temporary genetic difference, on which is superimposed non-genetic factors producing their parallel fluctuations. Clearly it is not due to a Lamarckian factor, for if this were concerned the divergence between the lines should increase as the number of generations of trained ancestry in line T increases. That this is not the case is shown by the column ratio T/C in Table 3.

Our conclusion is therefore that a genetic difference between the two lines in regard to learning facility appeared about generation 12, and disappeared, or greatly diminished, between generations 28–36. As we shall now describe, we have discovered two other independent genetic differences between the two lines which must be accounted for in some other way than by the accumulation of trained ancestry in the one line and not in the other. We are indeed faced with a difficulty inherent in all experiments requiring the independent maintenance through many generations of a control line for comparison with an experimental line. Whatever

care is taken to ensure that the two lines are genetically identical to begin with, mutations will in time destroy this identity.

A GENETIC DIFFERENCE IN BODY SIZE BETWEEN LINES T AND C

At about the 20th generation the fact had gradually impressed itself upon us that the rats of line T were almost consistently much larger and more vigorous than those of line C. The superior vigour of line T is manifested, among other things, by a much greater liveliness and eagerness when the lid of the cage is opened. This, however, may perhaps be accounted for by the greater tameness of the rats of this line owing to their handling when given their daily swim in the bath. This exercise, which continues from the age of 27 days till mating at 90+ days is also the only difference in the conditions under which the two lines live.

Systematic weighings were made in generations 25–28, the rats being weighed when 26, 75 and 125 days old. Line C rats, weighed at 75 and 125 days, were of course the untrained controls used for breeding. For the weights at 26 days it must be remembered that litters of more than six (which constitute the great majority of all litters) are reduced to that number within a few days of birth, except in a very few cases of second or later litters from large mothers which are left with seven or eight young to rear.

The mean weights with their standard errors are given in Table 4. It will be seen that line T rats are much larger at all three ages than those of line C. At 26 days the mean weight for line T exceeds that of line C by 31% of the latter.

Although it was obvious that this size difference was persisting, further weighings were made in generations 34–36. Fifty males and fifty females of both lines were weighed when 26 days old, with the result: T ♀♀ $53{\cdot}0 \pm 0{\cdot}7$ g., T ♂♂ $54{\cdot}8 \pm 0{\cdot}7$ g., C ♀♀ $38{\cdot}0 \pm 0{\cdot}7$ g., C ♂♂ $40{\cdot}3 \pm 0{\cdot}7$ g. In addition, eighty-five rats were weighed when 75 days old; the size differences between the two lines were again shown to be fully maintained—indeed, as at 26 days, they were slightly greater than in generations 25–28.

There remains to be discussed the possibility that this difference between the two lines is not genetic, but phenotypic only. It might be that the larger size of line T rats at maturity is due to their daily exercise in the tank, and that this accounts for the larger size of their offspring at weaning. The following experiments show, however, that the difference is inherited.

It is our custom to terminate the training of line C rats as soon as they have achieved the twelve consecutive runs to the dim gangway, so we have not many rats of that line which are still in training at the age of 75 days. In generations 34–35, however, owing to the slow rate of learning in these generations, to which we have already drawn attention, an unusually large number were still in training at that age. The mean weights of four females and five males still in training at that age were 136 and 187 g. respectively. Conversely, we reared a few rats of line T without training; at 75 days the mean weight of fourteen females was 175 g., and of eleven males the exceptionally high figure 247 g. This shows that giving the rats of line C

a daily swim in the bath, and depriving rats of line T of this exercise does not diminish the difference in weight between the two lines at the age of 75 days. Moreover, a few (16) offspring from these untrained rats of line T were weighed, and found to be above the mean weight of the line.

The fact that the size difference between the lines is genetic was confirmed by making a number of crosses between the two lines.* Ten such litters were obtained from the cross C ♀ × T ♂, yielding 28 ♀♀ and 28 ♂♂. The mean weight of the females at 26 days was 44·1 g., and of the males 45·2 g. These weights are intermediate between the weights of the pure lines. We have only three litters from the reciprocal cross T × C; at 26 days the mean weight of the nine female offspring was 51·9 g., and of the nine males, 53·1 g. This is actually heavier than the weights of the pure line T as shown in Table 4, but not significantly different from the weights of that line in generations 34–36, the contemporaries of the heterozygous litters in question.

Table 4. *Mean weights in grams, with standard errors, of rats of trained and control lines in generations* 25–28. *The figures in brackets are the number of rats weighed*

Age	Trained line	Control line
26 days	♀ (73) 50·5 ± 0·7	♀ (153) 38·0 ± 0·4
	♂ (72) 50·4 ± 0·6	♂ (139) 38·7 ± 0·4
75 days	♀ (65) 168 ± 3	♀ (65) 135 ± 2
	♂ (58) 217 ± 5	♂ (64) 184 ± 4
125 days	♂ (28) 311 ± 9	♂ (28) 257 ± 10

These results are consistent with dominance of the heavier weight combined with an inability of the smaller line C mothers to provide the intra-uterine, and possibly lactation, conditions to satisfy the full growth capacity of their heterozygous young which is so much greater than that of the usual offspring. In this respect these crosses resemble those between the horse and the ass, between species of *Cavia*, and between large and small races of rabbits, in all of which the mother has greater influence than the father on the body size of the offspring (Castle, 1941).

In order to elucidate further the genetics of the size difference between the two lines several of the young from the cross C ♀ × T ♂ were reared and mated together. Thirteen F_2 litters were thus obtained. At 26 days the mean weight of the thirty-seven females of these litters was 49·7 g., and of the thirty-nine males 51·7 g. These weights are only slightly less than those of the pure line T in generations 34–36 with which they were nearly contemporary.

There is no evidence of the segregation within these litters which is to be expected if the size difference between the lines is due to a single or few genes. Certainly there is no clear differentiation into two or three classes, nor any appearance of rats of the small size characteristic of line C. In the pure lines an occasional pathologically underdeveloped runt is found at weaning time, and rejected. One such runt occurred in the F_2 litters, weighing 26 g., its next smallest litter mate weighing

* These crosses were made specifically for the purpose of investigating the genetics of the size difference; none of the descendants of the crosses was used in the training experiment.

53 g. This rat has been omitted from the group. Except for it, not one of the F_2 rats is as light even as the mean weight of line C. Nineteen of them are heavier than the mean weight of line T, generations 34–36.

To obtain a rough quantitative comparison of the variability of these thirteen F_2 litters with that of the pure lines, the average deviation of each litter was found (without distinction between males and females), and also of fifteen litters in each of the pure lines. The mean average deviation of the F_2 litters is 2·30, of the line T litters 2·55 and of the line C litters 1·89. Expressed as percentages of mean weight, 100AD/M, these figures become: F_2, 4·56; line T, 4·81; line C, 4·90. The fact that the variability of the F_2 litters is no greater than that of the pure lines* is surprising on the ordinary genetical theory of the inheritance of quantitative characteristics. If it is assumed, nevertheless, that the size difference, being inheritable, is the expression of gene differences, one is forced to the conclusion that many genes are concerned, which is again surprising in view of the short time since the two lines were derived from the common ancestral pair.

On ordinary genetical theory there are two ways in which this genetic difference could have arisen. Either the original pair, from which both lines are descended, was heterogeneous for genes affecting size, and by chances of segregation genes favouring larger size have become concentrated in line T, and genes for smaller size in line C; or else mutations have taken place in one or both lines. It seems almost certain that segregation from the original pair must be excluded, for no difference between the two lines was noticed in the earlier generations; later, as shown by the figures quoted, the difference was far too great and constant to escape notice, not only by the eye but in the handling of the rats. Moreover, we happen to have some records of weights at 26 days old in generation 5, made for another purpose. For some reason, probably less efficient husbandry, the weights of the whole colony were much lower than in the later generations. The mean weight in line T (10 ♀♀ and 12 ♂♂) was 32·2 g., and in line C (21 ♀♀ and 23 ♂♂) was 30·3 g., giving a difference of $1{\cdot}9 \pm 0{\cdot}9$ g. in favour of T. This difference is not statistically significant, and in any case is of quite a different order of magnitude from the difference in later generations.

Comparison with the weights of Wistar albino rats given by Greenman & Duhring (1931), and by King (1915), suggests that the difference between our two lines has been caused by changes in line T, rather than in line C, during the period that has elapsed since their common origin. Greenman & Duhring record a large number of weighings spread over 4 years. Eight groups of males and females were weighed at 25 days old. The mean weights of the groups varied from 34·3 to 48·6 g. Thus even under the expert care available for the Wistar Institute colony there is great variation in weight at different times, which the authors show cannot be attributed, except in slight degree, to seasonal variation. The total mean weights (our calculation) for the 423 ♀♀ is 40·7 g., and for the 455 ♂♂, 41·3 g. To these

* In the routine reduction, a few days after birth, of litters of more than six to that number, we generally pick out the three largest and most healthy looking males and females; at least, conspicuously small and weakly rats are not included in the selection. In reducing these F_2 litters it was of course necessary to avoid scrupulously any selection. Had this been practised as in the case of the pure lines, their variability would probably have been even smaller.

figures about 2·5 g. should be added to make them comparable with our weighings at 26 days. Thus the Wistar Institute figures are intermediate between ours for lines T and C, but nearer line C. This applies also to the weights at 75 and 125 days. But our rats are unselected, the measurements applying to the whole colony, while those of Greenman & Duhring refer to a selected and specially cared for group, which are stated to be superior to the general run of the colony. It appears therefore that the Wistar rat in its own home is very similar in weight to our line C. The superiority of line T over the standard Wistar rat is also shown by the maximum weights attained. King obtained two males of over 400 g. (414 g. at 455 days and 437 g. at 485 days). In what appears to be about the same number of rats in generations 25–28 we had seven males in line T of over 400 g. Two of the largest of these, which were kept to enable them to reach maximum size, eventually exceeded 500 g. (503 g. at 350 days and 507 g. at 270 days).

An important question is—does difference in size directly affect rate of learning? If larger animals tend to learn more quickly than smaller ones, this would account for the slightly lower average level of training scores exhibited by line T for so many generations, though that would leave unexplained the more recent approximation of scores in the two lines in spite of the maintenance, and even increase, of the greater size of the rats in line T. So far as can be judged from variations in weight within each line, larger rats do not learn more quickly than smaller ones, but if anything the reverse. In the six groups furnished by the two lines in generations 26–28, the mean weight of quick learners (classes 1 and 2) was less than the mean weight of their whole generation in five cases. Not much stress can be laid on this, as the difference is of doubtful statistical significance. So far as the evidence goes, however, it is against the view that the smaller size of the control rats is responsible for their higher average training scores, and is in accord with the impression recorded by McDougall, Crew and ourselves (First Report) that weaker rats tend to make fewer errors than stronger rats.

However, there is little justification for applying to size differences of genetic origin conclusions drawn from the performance of rats of different weights within each line, which are certainly mainly phenotypic.

A CONCEALED GENETIC DIFFERENCE IN A COLOUR PATTERN GENE

Since our rats are albinos, this was only revealed by accident when rats from our stock were used for another experiment involving crossing them with piebald rats of the type known as 'hooded'. This revealed at once that lines T and C were carrying different allelomorphs of the gene concerned.

As the result of the work of Castle & Philips (1914), Castle (1916) and others, it is established that this gene has three allelomorphs: **H**, which gives a self-coloured rat, dominant to the other two allelomorphs **h′** and **h**; **h′** gives the pattern known as Irish, which in the homozygous condition is a rat fully pigmented except for a white spot, or thin white streak, on the belly. This is dominant to the allelomorph, **h**, which gives the hooded rat.

In Castle's well-known selection experiment on the pigmented area in hooded rats (**hh**) two rats from the same father appeared in his 10th generation which exhibited the colour pattern now known to be characteristic of the heterozygous Irish, **h′h**; this represents a considerably higher grade, that is to say, with a larger pigmented area than any which had previously appeared in Castle's stock. Experiments showed that these two rats differed from the rest of the stock, not in the modifying genes responsible for the ordinary variations of the extent of the hood in **hh** rats, but in the gene itself, which had mutated from **h** to **h′**.

Curtis and Dunning (1937) also obtained two independent mutations of the gene **h** to its dominant **h′** in unrelated stocks.

There are therefore three records of this mutation. Apparently we have to add a fourth.

The strain of hooded rats used for crossing with our albino stock varied but little from Castle's grade 0, the typical hooded pattern. The members of the strain actually used for the crosses ranged from grade $-\frac{1}{2}$ to grade 0. The colour of the pigmented area is black.

In generation 19, two males of our stock, one from line T and one from line C were mated in succession to the same hooded female. The C male gave a typical hooded F_1, all about grade 0, while the F_1 from the T male were all about grade $+4\frac{3}{4}$, which is typical of the heterozygous Irish pattern. This unexpected evidence of a genetic difference between the two lines was followed up in generations 24–26 in which fifteen C rats and thirteen T rats were tested by mating with hooded rats. The fifteen C rats produced 125 F_1 young, ranging from grades $-\frac{1}{2}$ to $+1$. The thirteen T rats produced 136 F_1 young, all between grades $+4$ and $+5$. Matings between the F_1 rats derived from line T, the colour pattern of which indicated the genetic composition **h′h**, yielded approximately the expected proportion with the pattern of the homozygous Irish, **h′h′**.

This constant difference between the rats of the two lines, picked at random from four generations, is sufficient to show that line T is homozygous for **h′** and line C for **h**. Since the difference was first discovered in generation 19, we are not in a position to say definitely whether it arose as a mutation in one line, or whether both allelomorphs were present in the original pair from which both lines owe their origin. The standard Wistar rat is however known to be homozygous for **h**, and as our stock has had no outside blood introduced into it while it has been in our hands, and we are assured that this applies also to the many generations which elapsed from the time it was obtained from the Wistar Institute and the time it came into our possession, it is highly probable that a mutation from **h** to **h′** occurred in an early generation of our line T.

GENETIC INDEPENDENCE OF THE DIFFERENCES IN SIZE AND COLOUR PATTERN

Since lines T and C carry different allelomorphs of the pattern gene **H**, it seemed possible that the difference in body size might be an expression of the same gene difference. To test this, F_1 heterozygotes from crosses between the two lines were

mated to hooded rats to introduce the pigment factor, and the offspring of these matings were bred together, giving pigmented **hh**, **h'h** and **h'h'** rats (as well, of course, as albinos of unknown genotype). A total of 156 of the pigmented rats were weighed when 26 days old, showing no significant difference between the three genotypes.

The **h** and **h'** allelomorphs are therefore not responsible for the difference of body size between the two lines. These now differ by at least two genes (for hooded and weight), or more likely by many if, as appears probable, the difference of weight is an expression of many genes.

SIGNIFICANCE OF THE DISCOVERED GENETIC DIFFERENCES BETWEEN THE TWO LINES

For our immediate problem the important fact is that since at least generation 19, lines T and C have not been genetically identical for factors which cannot be related to the effects of training. Since our rats are albinos, mutation in colour or pattern genes would not have been detected but for the accident that some of the rats were used for another experiment which involved crossing them with a pigmented form. We have no way of telling what other somatically invisible mutations may have taken place, nor whether these may directly or indirectly influence the rate of learning.

As we have shown, between about generations 12–28 the mean training scores of line T were lower than in line C—almost consistently so when individual generations are compared, and quite consistently for the larger groups of four generations. Later this difference disappeared. In view of the other genetic differences which have developed between the two lines, it seems reasonable to attribute this variation in training scores, maintained over many generations, also to mutations.

ABSENCE OF PARENT-OFFSPRING CORRELATION FOR RATE OF LEARNING

Line C is not available for testing parent-offspring correlation, since the parents are not trained. Line T was tested for this correlation by correlating the mean classes of litters with the mean classes of their mid-parents—that is to say, the mean class of the two parents. This was carried out for the fifteen generations 18–32, beginning therefore with the first generation reared in the new cages and on the new diet as described in our Second Report. The offspring furnished by these fifteen generations comprise 121 litters and 696 rats.

The mean class of the mid-parents is $4{\cdot}79 \pm 0{\cdot}17$, and of the litters $4{\cdot}19 \pm 0{\cdot}13$. The parent-offspring correlation is $+0{\cdot}064 \pm 0{\cdot}091$. Therefore no genetic differences in learning rate between members of the line T are disclosed.

To test this further, a comparison was made between the offspring of the quickest and slowest learning parents. Within the fifteen generations there are eight matings in which both parents were quick (class 1 or 2). There are nine matings of slow parents (both class 7 or over). The total number of offspring is fifty-one in both groups. The mean class of offspring from the quick parents is $4{\cdot}29 \pm 0{\cdot}37$ and from the slow parents $4{\cdot}20 \pm 0{\cdot}41$. This test therefore also fails to disclose any genetic differences, even between the quickest and slowest rats within line T.

Crew (1936), on the other hand, found a parent-offspring correlation of +0·3, making it 'certain that genetic factors are largely concerned in determining the score that a given individual shall make'. It must be noted, however, that, granted the existence of genetic factors affecting rate of learning, Crew's method of breeding favoured the production of individuals and pedigrees tending to homozygosity for such factors. The total population which yielded the correlation coefficient was composed of six lines, maintained without interbreeding from the second generation. Only two of these lines, however, survived to the end of the experiment (eighteen generations). These two lines provided 586 (line A) and 564 (line B) of the total 1449 rats. Crew states that the average score of line A rats is significantly lower than of line B. Moreover, within each line he practised mating quick learners with quick, and slow with slow.

Our method of random mating does not tend to segregate factors for quick or slow learning into individuals or pedigrees.

Between generations 28 and 33 we made a succession of brother-sister matings, but without regard to the individual scores. If significant genetic differences exist, this should result in splitting the line into sublines of different average training scores. As no evidence of such differentiation was obtained, we reverted from generation 34 onwards to our old method of mating, which excludes brother-sister matings as far as possible.

The conclusion therefore is that our line T is genetically homogeneous for genes affecting rate of learning, or else that if genetic differences are present, their influence on the training score is so small compared with that of non-genetic factors as to fail to produce a significant parent-offspring correlation.

INITIAL PREFERENCE FOR THE BRIGHT OR DIM GANGWAY

In his Third Report (1933, in collaboration with Rhine) and his Fourth Report (1938), McDougall introduced another test of the effects of ancestral training. This is the initial preference of the rats for the bright (B) or dim (D) gangway, before any experience of the shock on B. His practice was to give his rats, on the day preceding the beginning of training proper, six runs in the tank with the light alternating between left and right in the usual way, but with no shock on B. He found that the earlier generations of his trained lines, and his batches of control rats, showed a slight preference for B on this day (zero day). In the Third Report he produces striking evidence of a change in this respect. The lines concerned are (1) the principal line, TR, on which the conclusions from his experiment are mainly based; (2) a line selected for slow learning, WC (WH) in which the slowest half of each generation was used for breeding; (3) a line, WC (BH_2) similarly selected for quick learning.

In the 16th generation in both selected lines there was a change from the previous slight average excess of choices of B on zero day to a large excess of choices of D (250 D–140 B in one line, and 127 D–77 B in the other) (1938, Tables VII, X, XI).

A similar, but very slight, change occurred in generation 32 of the main line (i.e. TR 32); this was much more marked in TR 33. His Fourth Report shows that the altered behaviour was maintained, on the whole, in the later generations of all

three lines. McDougall italicizes his opinion that '*this preference* (for D, before experience of the shock on B) *has thus become one of the leading evidences which I adduce in support of the Lamarckian interpretation*' (1938, p. 325). Because of the importance that is likely to be attributed to this conclusion, we have made a detailed analysis of the evidence for it. We believe that this analysis demonstrates the invalidity of his reasons for invoking the Lamarckian factor, or indeed any genetic factor at all.

There can be no doubt of the reality of the change from an average preference for B on zero day in the earlier generations to one for D in the later generations (1938, Tables VII, X, XI). The question is—was it due to a genetic change, whether Lamarckian or not, or may it with equal reason be attributed to some external factor? There would be strong grounds for postulating such a factor operating similarly on all three lines if the change in the three lines took place synchronously. If, however, it occurred at different times in the three lines, especially if these times bore some relation to the number of generations during which the lines had been in training, there might be some justification for McDougall's Lamarckian interpretation. It is a striking fact, however, that when the change appeared one line had been trained for some twelve years, the other two for six.

We are left therefore with the question—did the change appear synchronously in the three lines or not? In discussing, and rejecting, the possibility that the change might be the result of some external factor, McDougall stresses that the change was *not* synchronous in the three lines. This, however, depends on his location of the change in TR 32 and not in TR 33, and also in generation 16, not in generation 15, of the two selected lines WC (see his dates of training these generations, 1933, p. 230). If, however, we locate the changes in TR 33 and generation 15 of the two selected lines, then the change is as nearly synchronous as different dates of training permit. A decision on this point is essential to McDougall's argument, and depends on whether the figures for the generations in question are to be considered fluctuations in the earlier series of generations with its average preference for B, or in the later series with its average preference for D.

On these grounds there appears to be no justification for McDougall's location of the change in TR 32 rather than in TR 33. The zero-day choices in TR 32 (42 rats) were D 137–B 115, or 54·4% D. We can find* from 1938, Table VII, that the total zero-day choices for the eighteen generations before TR 32 for which records are given (TR 13–31, 345 rats) are D 1005–B 1065, or 48·6% D; for the twelve generations after TR 32 (TR 33–44, 313 rats) the choices are D 1141–B 737, or 60·8% D. The question therefore is: Is the 54·4% D of TR 32 to be considered as a chance fluctuation under conditions giving the earlier 48·6% D, or a chance fluctuation under conditions giving the later 60·8% D? McDougall implies that he accepts the latter, for this is necessary if the change to increased preference for D is to be located in that generation. The figure 54·4% is however almost exactly midway between the earlier 48·6% D and the later 60·8% D. On this criterion therefore there is no

* All calculations of percentages are our own. McDougall makes practically no attempt at statistical analysis of his figures.

justification for considering it, as McDougall's view necessitates, a member of the later rather than of the earlier series.

Moreover, if we examine in detail the generations before and after TR 32, we find that that generation falls within the range of variation of both series. Two of the eighteen generations of the earlier series (with twenty-three and fourteen rats respectively) show a greater preference for D than do the forty-two rats of TR 32, and two of the later series (twenty-nine and thirty rats) show a smaller preference.

We conclude therefore that McDougall's own figures show no justification for classing TR 32 with the later rather than with the earlier series of generations, and therefore for locating the change in that generation rather than in TR 33. TR 32 falls within the range of variation of both series and is intermediate between the average choices of D and B in the two series.

We must now examine McDougall's justification for locating the change in generation 16, rather than in generation 15, of the two selected lines, for to establish the synchrony of the change in the three lines we must disagree, not only with McDougall's location of the change in TR 32, but also with his location of it in generation 16 of the two selected lines.

In these two lines, the generations before 15 are common to both, the line WC (WH), selected for slow learning, being split in generation 14 into two sublines; selection for slow learning was continued in the old WC (WH), and reversed to selection for quick learning in the other, now designated WC (BH_2).

Generation 16 (1938, Tables X, XI) certainly shows a pronounced change from the former average preference for B, giving D 250–B 140 in the one line, and D 127–B 77 in the other. The preceding generation 15, however, also shows, although less markedly, a decline in the former preference for B, the two lines together giving an equality of choices (D 168–B 168).

In deciding whether the change in these lines is to be located in generation 16 (as McDougall's argument requires) or in generation 15 (which would make it synchronous with TR 33) we have again to decide whether generation 15 falls most naturally within the range of variation of the earlier generations before the change took place, or of the nine later generations after it had taken place. Examination of these later generations, 16–24, in the two lines shows that generation 15 falls within the range of variation of these later generations, for in both lines three out of the nine generations show a considerable preference for B, and therefore a much greater deviation from the average preference for B exhibited by the group of nine generations as a whole than does generation 15 with its equality of choices.

To sum up our discussion of the grounds on which McDougall denies synchrony to the change in the three lines, on which denial he bases his exclusion of an external factor and attributes it to the Lamarckian factor: there is no doubt that from TR 33 and generation 16 of the two selected lines, onwards, all three lines show a marked average preference for D, compared with an average preference for B in the earlier generations. If we accept McDougall's contention that the change began in TR 32, WC (WH) 16 and WC (BH_2) 16, then it began nearly a year earlier in TR than in the two selected lines, thus excluding its explanation by the action of an external factor

operating in common on the three lines. But if we locate the change in TR 33 and in generation 15 of the two selected lines, as we contend it is at least as reasonable to do, then all three lines show it in the first generation whose training began later than September 1932.

In further discussion, and rejection, of the possibility that the change was due to an external factor, McDougall refers to the fact that in 1932, in which this new, decided preference for D began to be manifested, it became necessary to build new tanks to replace the old pair which had been in use for some ten years (1938, p. 325). The new tanks proved to offer to the rats a less difficult task than did the old ones, less difficult by approximately one third. The illumination of the gangways and of the walls of the passage leading to them was rather brighter than in the old tanks, and the curved end of the tank (facing the rats at their starting point) was painted in vertical stripes of black and white (1933, p. 218). Unfortunately we are not told which of the generations which began their training in 1932 was the first to be trained in the new tanks. McDougall, however, presents evidence that the change in the zero-day preferences for D or B, which took place about the time of the introduction of the new tanks 'cannot be attributed to the change from the old to the new tanks, save in some very slight degree' (1938, p. 327). The evidence consists of several batches of control rats with untrained ancestry, comprising a total of 106 trained in the old, and 103 in the new, tanks. The former gave zero-day choices D 302–B 334 (47·5% D), and the latter D 313–B 305, or 50·6% D.

The fact that these control rats in the new tanks show only such a slight preference for D is taken by McDougall as proof that the change from the old to the new tanks cannot be responsible for the much larger increase in preference for D exhibited by the three trained lines in the new tanks. But again this conclusion depends upon the exclusion of the small preference for D shown by the control rats in the new tanks as a chance fluctuation from the larger preference for D to be expected in the new tanks if these were responsible for the change in the trained lines. But, as we have seen, six out of the eighteen generations of the two selected lines in the new tanks show not only a smaller preference for D than these controls, but actually a preference for B. For the third time therefore a group of rats whose performance McDougall relies on for denying the operation of an external factor (this time, specifically, the new tanks) is found to fall within the range of variation of the groups from which it is necessary to exclude it if McDougall's contention is to be substantiated.

We may also draw attention to another fact adverse to McDougall's interpretation of the cause of the change to a preference for D after transference to the new tanks. If, as he maintains, it is the Lamarckian effect of previous generations of training, it might surely be expected that with continuance of the training later generations would show a further increase of preference for D. This can be investigated from McDougall's records. In his Fourth Report he gives the figures for thirteen generations of TR, and nine of each of the two selected lines, after the change from an average preference for B, to an average preference for D. Leaving out the middle generation of each line to get equal halves, we can compare the first six with the last six generations of TR, and the first four with the last four generations in each of

the two selected lines. The first halves, consisting of generations TR 32–37, WC (WH) 16–19 and WC (BH_2) 16–19, a total of 447 rats, show 56·5% choices of D. The second halves, TR 39–44, WC (WH) 21–24 and WC (BH_2) 21–24 (371 rats) show 57·0% choices of D. The combined lines show therefore no significant increase of preference for D with further accumulation of trained ancestry.

Summing up the whole of the foregoing analysis of the evidence, we cannot accept McDougall's conclusion that the change from an average zero-day preference for B in the earlier generations to an average preference for D in the later generations, was a Lamarckian effect; or, indeed, that it was a genetic change of any kind. The evidence is equally compatible with, or even more favourable to, the conclusion that it made its appearance in the first generations of the three trained lines which began their training later than September 1932. This points to the operation of an external factor, which continued to operate with equal force throughout the later generations, rather than to a Lamarckian factor which produced its effect suddenly and almost simultaneously on the three trained lines, in spite of the fact that one of them had been in training for about twice as many years as the other two, and which failed to increase its effect after many more generations of training. While it is not necessary for our argument to identify the external factor responsible for the change, we do not think that McDougall has produced any valid grounds for his contention that the factor in question could not be the transference from the old to the new tanks.

We cannot present figures of the initial preferences for D or B quite comparable to McDougall's, owing to the fact that on the first day (McDougall's zero day, before training proper begins) we, like McDougall, give our rats six runs in the tank to give them a preliminary acquaintance with it, but on this day we use neither the light nor shock, whereas McDougall used the alternating light but no shock. On zero day, therefore, McDougall's rats could take the bright gangway without receiving the shock. Our rats experience the shock on the first occasion (usually on the first day of training proper) that they choose that exit. As, however, only a very few of the exceptionally quick learners are avoiding B on the later runs of the first day as a result of experience of the shock on B on an earlier run on that day, the ratio of the choices of B and D on our first day of training are practically equivalent to McDougall's figures for zero day.

Table 5 gives the ratios of the choices of B and D in the four runs of the first day of training proper, dividing the total experiment into four successive quarters. This table shows a general parallelism between the changes in per cent choices of D on the first day of training, and the changes in training scores as shown in Table 3, column *Mean class*. For (1) in both, the changes run parallel in the two lines, T and C, (2) in the early part of the experiment the choices of D are low, followed by a long period when the choices of D are much higher; in the last quarter of the experiment there is a return to the original low percentage of choices of D. It will be noted again that if we had had no control line, and had stopped the experiment at about generation 27, the increasing preference for D on the first day of training could have been interpreted as a Lamarckian effect.

It will be noted that McDougall's figures and our own both show an inverse correlation between initial choices of D and training score. Generations with a higher initial choice of D learn with fewer errors than generations showing a lower initial choice of D. This is not a spurious correlation, due to the fact that the number of runs to B (errors) on the first day is included in the final score, for the extreme differences in the per cent choices of D in Table 5 represent much less than a difference of one choice of D in the four runs; this is a negligible contribution to the differences in mean training scores of different generations. This suggests the influence of a common factor on the initial preference for B or D, and the total number of errors made before learning.

Table 5. *Choices of D on the first day of training*

Generations	Line T		Line C	
	No. of rats	% of choices of D	No. of rats	% of choices of D
1–9	192	47·14	192	45·05
10–18	410	54·02	378	48·54
19–27	436	59·29	433	52·77
28–36	390	47·88	396	43·94

Expectation, in absence of bias, is 50 % D.

SUMMARY

This experiment, to test McDougall's conclusion that the effects of training are inherited, has now been carried on for thirty-six generations, involving the training of 2827 rats. The present position of the problem raised by McDougall may be summarized as follows:

Neither our own experiment, nor that of Crew, shows any evidence of increasing facility in learning attributable to trained ancestry. McDougall's claim that the progressive decline in the number of errors which he found in successive generations of trained rats is an example of Lamarckian inheritance cannot be maintained in face of the facts (*a*) that he did not keep a control line, (*b*) that we have found a progressive decline in our trained line similar to McDougall's, but this was paralleled by the control line; moreover, after about twenty-eight generations, the number of errors progressively increased again in both lines.

McDougall's further argument from the change from a zero-day preference for the bright gangway in earlier generations to a preference for the dim gangway in later generations is invalid; it is shown, from his own figures, to be capable of a different explanation.

The discovery of genetic differences in colour pattern and body size between our trained and control lines, presumably due to mutations, emphasizes the difficulty of interpreting genetic differences in facility of learning, even if they should occur, as due to the Lamarckian factor.

The experiment is being continued.

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STUDIES IN THE RESPIRATION OF *PARAMECIUM CAUDATUM*

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(With Three Text-figures)

The lack of specific enzyme studies on protozoan material may be attributed mainly to manipulative difficulties. Burge & Williams (1927), in their substrate-utilization study on *Paramecium caudatum*, stated the position as follows: 'The most difficult part of this investigation was the raising of these organisms in sufficiently large quantities and in fairly pure cultures.' The slow advance of our knowledge of the nutrition of most types of Protozoa has left the position substantially unaltered. A few cultures of Protozoa on defined medium in the absence of other living organisms have been established (Doyle, 1943). Notably *Chilomonas* (Mast, Pace & Mast, 1936; Hutchens, 1939) and *Tetrahymena* (Kidder & Dewey, 1945) have been grown in a culture suitable for metabolic studies; many types have not been cultured free from their living food organisms, e.g. *Amoeba* and *Spirostomum*, and the bacteria-free culture of *Paramecium* of Johnson & Baker (1942) has a division rate so low as to render its use in metabolic studies impracticable.

With 'wild' cultures of Protozoa, the first problem in preparing animals for experiment is one of lowering to an insignificant level the percentage of contaminant bacteria associated with the animals; failure to do this invites criticism of some earlier work in the field. The usual method is washing by repeated centrifugation (Lund, 1918; Pitts, 1932, etc.), but in our hands this procedure was found to damage a large percentage of the animals, and it became necessary to discover some other means of purifying the suspension. Root (1930) collected *Paramecium* at the top of a glass cylinder by use of the animals' negatively geotropic reactions. The method described in this paper is one involving electrically directed migration up a 'sterile' column of salt solution. This treatment leaves the animals intact and also produces a certain degree of physiological segregation. However, any experiments with organisms separated from non-sterile cultures should include bacterial blanks with animals removed (Boell & Woodruff, 1941) or killed thermally (Peters, 1927). This precaution, too, has often been omitted (Leichsenring, 1925).

A more difficult problem is that of measuring the metabolism of the small amount of material which thus becomes available for study. Even though the cultures may be luxuriant, enormous numbers of animals are needed to investigate respiration or enzyme composition by ordinary manometric techniques. An examination of the experimental figures given by various workers who have used Warburg manometers, presumably of normal types (Mast *et al.* 1936; Pace & Belda, 1944*a*, *b*; Burt, 1945;

Pace, 1945, etc.) would indicate that the oxygen uptakes recorded were below the sensitivity of the methods employed. The most successful studies have been carried out with ultra-microrespirometers which demand less protozoan material and which give adequate readings within permissible experimental time.

In some cases the experiments have been performed to demonstrate the utility of a particular instrument (Holter, 1943; Zeuthen, 1943) rather than to obtain information on the metabolism of Protozoa (Boell & Woodruff, 1941; Boell, 1945). In this paper is described a Cartesian diver respirometer of 'macro' dimensions which, though lacking the precision of the Carlsberg apparatus, was more practicable and useful in view of the accuracy necessary for the problem under investigation.

For many years the belief that *Paramecium* respiration was entirely mediated by an unusual route, cyanide-stable, has persisted. The demonstration of the presence of cytochrome (Sato & Tamiya, 1937) and of cytochrome oxidase (Boell, 1945) in *Paramecium* has shed new light on this. Since succinic dehydrogenase is one of the two animal dehydrogenases known to react directly with cytochrome and cytochrome oxidase, we have studied its occurrence in *P. caudatum*.

METHODS

Culture of the organism

The *P. caudatum* used in this study was supplied, in admixture with *Euglena* and *Chilomonas*, by Prof. Agar of the Department of Zoology, University of Melbourne. A clone free from the other Protozoa was established. The culture medium consisted of 5 ml. of Osterhout solution (Leslie, 1940) and 5 ml. of 20% Vegemite suspension in 1 l. of distilled water. The Vegemite is a yeast concentrate manufactured by the Kraft-Walker Cheese Co. Pty. Ltd., Australia, and served to support a rich bacterial flora upon which the Protozoa fed. The medium was distributed in 150 ml. aliquots in 250 ml. Florence flasks. The inoculum was 10 ml. of suspension from a 2- or 3-day culture; all cultures were grown at 28° C., at which temperature optimum division rate occurred.

The population reached its maximum density of one to two thousand animals per ml. on the fourth day (Fig. 1). The organisms used in experiments were always culled from 2- or 3-day cultures, where growth was approximately logarithmic. Counts were by the Sedgewick-Rafter cell (Hall, Johnson & Loefer, 1935), and the number of fields which it was necessary to count to give a precision of 10% was determined statistically. As a routine, thirty fields were counted.

Preparation of the suspension

The fact that a slight electric current causes a reversal of ciliary beat (Jennings, 1904) and a migration to the cathode formed the basis of our 'electromigration' apparatus (Fig. 2). This consisted of a 100 ml. Erlenmeyer flask, to the top of which was fused 15–20 in. of $\frac{3}{4}$ in. glass tubing. An electrode was inserted at the bottom of the flask either by fusing in a short length of tungsten wire, or by fixing, by means of sealing wax, a piece of nichrome wire into a short, tapering side-arm of $\frac{1}{4}$ in. glass tubing. This lower electrode was connected through a mercury pool to the anode of

a dry cell. The other electrode, attached to the cathode, was a hook of nichrome wire at the top of the apparatus. The flask was filled with 100 ml. of wild culture, filtered

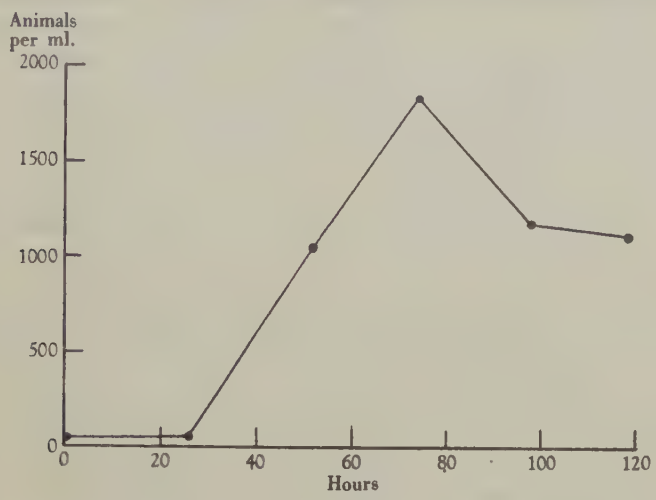

Fig. 1. Growth curve of *Paramecium caudatum* in Vegemite-Osterhout medium.

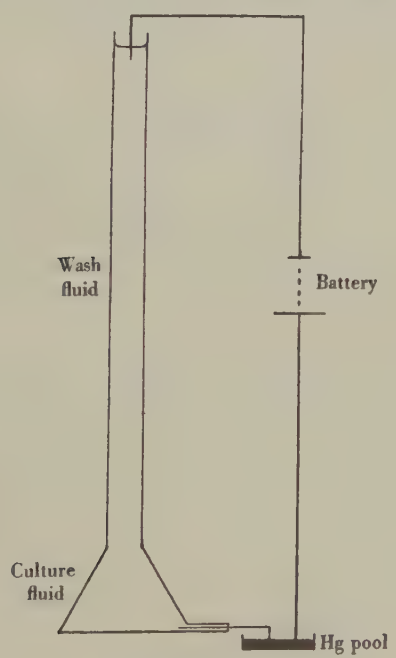

Fig. 2. Electromigration apparatus.

through cotton-wool to remove mould. The column was then filled cautiously with the wash fluid so that mixing did not occur. The wash fluid consisted of 1:100 Osterhout solution, in which dilution the animals were found to survive longest.

In a time varying with the voltage applied and the age of culture, the majority of animals in the bottom flask migrated through the clear wash solution and congregated in the top 25 ml. of fluid. It was thought that some physiological segregation occurred as dividing forms did not appear at the top of the column, and in old cultures the number of animals which failed to migrate was greatly increased. Organisms from 2- and 3-day cultures always completed each migration in less than 20 min. Those from 4- and 5-day cultures needed from 20 to 60 min. and gave less dense collections of migrated animals; 5-day cultures took over an hour. The animals in the top 25 ml. of several migrators were withdrawn, combined and subjected to a second migration before being used in an experiment; 22 V. was always used as the potential difference between the two electrodes (Table 1).

The suspension of animals withdrawn from the top of the apparatus after the second migration was centrifuged in 10 ml. tubes for 30 sec. at 1000 r.p.m., and the supernatant liquid removed by suction. This centrifugation did not damage the animals as judged by microscopic examination. The final suspension, containing $2–6 \times 10^5$ organisms per ml., was transferred to a ground-glass homogenizer (Humphrey, 1946) and an aliquot withdrawn for counting; in the remainder, cell structure was completely destroyed by homogenizing for 2 min. at 0° C. Homogenizing at room temperature, or for 3 min., yielded a preparation which did not respire. Aliquots were placed in small tubes and buffer, substrate, inhibitor, etc., added; 10 μl. of the mixture was then pipetted into the appropriate diver.

Manometric technique

The general plan of apparatus followed that of Linderstrøm-Lang (1937), Linderstrøm-Lang & Glick (1938) and Boell, Needham & Rogers (1939). These were the only papers available until towards the end of the investigation when micro-films were obtained of the complete papers of the Carlsberg Laboratory (Holter, 1943). The apparatus contained six chambers, with a 1 m. manometer scale, and pressure in the manometer was controlled by means of a 20 ml. and a 2 ml. syringe.

Following the method of Boell *et al.*, calculation of the diver 'constant', i.e. μl. gas change in the diver per cm. discursion on the outer limb of the manometer scale, was made by the formula which these workers arrived at by modifying the usual equation for the Warburg manometer. However, it became apparent that there was a serious error in the modification proposed by these workers for the fact that 'only the open limb of the manometer is read'. They state, 'It was experimentally found that in the plan of apparatus finally adopted, a rise of 10 cm. in the open limb of the manometer always implied a rise of 6·61 cm. in the closed limb. The equation finally used, therefore, was as follows:

$$K_{CO_2} = \frac{(V-4) \times \frac{273}{293} + 4 \times 0{\cdot}86}{10{,}350} \times 0{\cdot}661.'$$

If, on raising the level in the outer limb 10 cm., the inner limb level rises 6·61 cm., the difference between the two levels is 4·39 cm. and obviously, this difference is

the effective pressure acting on the surface of the flotation medium. The factor applied should be, therefore, not 0·661, but 0·439; or, more generally, if x is the rise in the closed limb for every cm. rise in the open limb, the factor to be applied to the constant obtained by the Warburg equation is $(1-x)$. Then K_{CO_2} multiplied by the manometer discursion in the open limb during the experiment gives the alteration in gas volume within the diver, expressed in μl. CO_2. It seems improbable that this error was really entertained by Boell *et al.* (1939), and is other than an inadvertent misstatement, but until its formal correction, it must cast some confusion on the results of Boell & Woodruff (1941), Boell (1945), and any others who have 'Followed closely the descriptions given by Boell, Needham & Rogers (1939)' (Clark, 1945).

It follows that the larger the gas space between the surface of the flotation medium in the chamber and the water-level in the inner limb of the manometer, the more nearly will the rise in the inner limb of the manometer approximate to that in the outer limb, and the smaller will the difference in levels, and hence the factor, become. The decrease in factor correspondingly occasions a decrease in the constant K, i.e. increases the sensitivity of the instrument, since a 1 cm. change on the manometer scale indicates a smaller gas change within the diver. For this reason, the gas space between the closed limb water-level and the surface of the flotation medium was increased in our apparatus by the insertion of a 250 ml. Erlenmeyer flask into the closed circuit. This lowered the factor from 0·82 to 0·54 (i.e. without the extra gas space, a rise of 10 cm. on the open limb caused a rise of 1·8 cm. in the closed limb; after insertion of the gas space, a rise of 10 cm. in the open limb caused a rise of 4·6 cm. in the closed limb), and for the experiments quoted here, an instrument with a factor of 0·54 was used.

These considerations with regard to the method of calculation do not apply to the methods adopted by the Carlsberg school, since those workers calculate the gas exchanges in a more fundamental manner, i.e. by reducing the gas space between the top of the flotation medium and the manometer fluid to a minimum, and working out a constant based on the specific gravities of the oil seal, the glass of which the diver is made, the flotation medium, etc. In this case, the applied pressure is fully effective since the gas space is small, i.e. the factor is unity.

Our divers were much larger than any others reported in the literature, having a total volume of 40–60 μl. The neck diameter was 1–1·5 mm. and the neck length 5–7 mm. Pipettes were graduated opsonic pipettes, drawn out by the technique of Holter (1943) to capillary tips. The bottom drop in the diver was 10 μl., and alkali, oil and neck seals about 1 μl., though these were not measured. The volume occupied by these was estimated by observing the fraction of the neck occupied by liquid at the equilibrium position. The use of large divers and large volume of experimental fluid are undoubtedly undesirable, since the first lowers the sensitivity, and the second introduces the possibility of a diffusion effect through the liquid. That a diffusion lag did exist was indicated by the fact that divers containing only 5 μl. of suspension generally exhibited a rate of gas consumption 10–15% higher than a corresponding diver containing 10 μl. at the end of the first hour, though the

results more nearly coincided for the second hour. However, through greater ease of reading large manometer discursions, and higher accuracy of pipetting 10 μl., it was found that greater consistency of duplicates was obtained using the larger fluid volume. Therefore, this was adopted as a standard procedure. Results may be regarded as precise to 10%.

Decinormal sodium hydroxide was used as an alkali seal (Linderstrøm-Lang, 1943), and kerosene-paraffin mixture as an oil seal (Linderstrøm-Lang & Glick, 1938). The flotation medium was, in early experiments, saturated ammonium sulphate, but when the paper of Holter (1943) finally became available, a change was made to the medium recommended by this author.

RESULTS

Effect of voltage on time of migration

In the method of electromigration described above for collecting the organisms, it was stated that migration was carried out with a potential difference of 22 V.; the adoption of this figure followed a consideration of the effect of voltage on the time of migration (Table 1).

Table 1. *Effect of voltage on time of migration*

2-day cultures were used.

Voltage	Current (μA.)	Time of first migration (min.)	Time of second migration
0	0	30	No migration
7	200	28	53 min.
15	580	15	17 min.
22	920	8	16 min.
45	2250	Animals dead	—

In the absence of electrical stimulation, animals failed to achieve a second migration, whereas a high voltage killed the animals. The voltage which we adopted combines speed of migration and lack of physical injury to the animals (which were capable of living and dividing indefinitely after the treatment).

Effect of pH *on the endogenous respiration*

The effect of pH on the endogenous respiration of the homogenate was studied in the presence of phosphate over the range 3·2–8·5. As Fig. 3 shows, there is an optimum in the range 6·6–7·6, with the rate falling off very rapidly in more alkaline reactions. On the acid side of the optimum, however, respiration shows more stability, falling off only gradually to pH 3·7. This may be correlated with the wide pH tolerance of the animals in culture medium.

The experiments reported in this paper were subsequently carried out at a pH of 7·2.

Effect of inhibitors and methylene blue on endogenous respiration

Table 2 shows the effect of cyanide, azide and methylene blue on the endogenous respiration of the homogenate.

Inhibition by cyanide was 56%, and by azide 44%. Azide might be expected to be less effective at pH 7·2, but attempts to demonstrate its effect at pH 4·2, in which region it has been shown to have its maximum effect on the oxygen consumption of yeast cells (Keilin, 1936), were unsuccessful, owing, probably, to the volatilization of the free acid. It might be noted here that no attempt was made to add suitable concentrations of cyanide to the alkali seal of the diver neck, as is the custom in manometric experiments involving cyanide (cf. Boell, 1945; Clark, 1945).

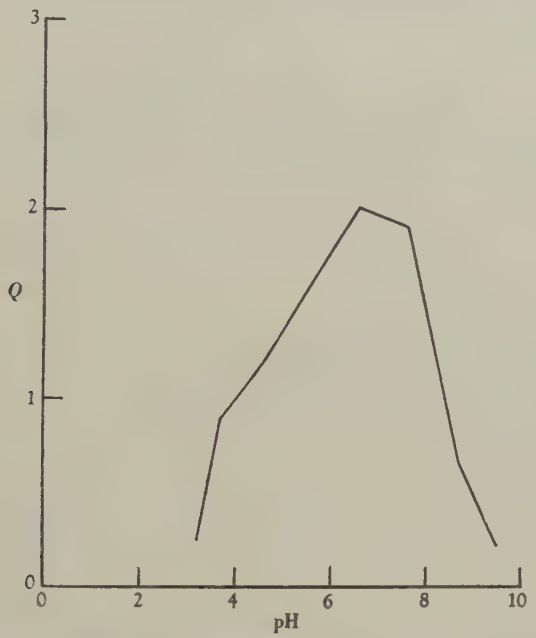

Fig. 3. Effect of pH on the endogenous respiration in the presence of 0·03 M-phosphate.

Table 2. *Endogenous respiration*

The *Q*'s are given as μl. $O_2/10^4$ organisms/hr.; 0·01 M-cyanide and azide were used, and 0·5 mg./ml. methylene blue. Cyanide and azide were neutralized before use.

	Q
Homogenate	1·9
Homogenate + KCN	0·8
Homogenate + NaN_3	1·1
Homogenate + methylene blue	1·9

These workers state that they used the mixtures recommended by Krebs (1935). However, since the mixtures recommended by Krebs for use in Warburg manometers were of one to two molar strength, compared with the decinormal alkali recommended by Linderstrøm-Lang (1943), there is the possibility of error due to the difference in osmotic strength between alkali seal and the experimental drop. Also, in view of the work of Riggs (1945), emphasizing the inadequacy of any simple theoretical analysis to predict conditions required in experimental assemblies to prevent alteration in cyanide concentrations due to distillation into alkali, it seems

doubtful whether the adoption of the mixtures suggested by Krebs would contribute effectively to the maintenance of a given cyanide concentration. It was tentatively decided not to add cyanide to the alkali at all, especially in consideration of the low alkali concentration, the temperature, and the experimental period which we employed. This procedure was justified by the observation that the degree of inhibition did not alter during the first and second hour periods.

Methylene blue did not increase the endogenous respiration, a finding which might be attributed to a sufficiency of carriers already present in the system for the low respiratory rate, or perhaps a lack of substrate.

Effect of succinic acid on respiration

Table 3 presents the effect of inhibitors on oxygen consumption in the presence of succinic acid, with and without the addition of methylene blue.

Table 3. *Oxidation of succinic acid*

Same conditions as for Table 2, with 0·05 M-succinate and 0·08 M-malonate.

	Q
Homogenate	1·9
Homogenate + succinate	4·3
Homogenate + succinate + KCN	1·8
Homogenate + succinate + NaN_3	2·0
Homogenate + succinate + methylene blue	6·0
Homogenate + succinate + methylene blue + KCN	4·8
Homogenate + succinate + methylene blue + malonate	2·0

Succinic acid caused a large increase in oxygen uptake which was sensitive to cyanide and azide, as would be expected if succinate oxidation were proceeding through the succinoxidase system. In the presence of methylene blue, cyanide did not inhibit the succinate oxidation, while methylene blue alone caused a higher rate of succinate oxidation. Further evidence that a succinic dehydrogenase similar to that found in other animal tissues was present in the homogenate is given by the action of malonate. This inhibitor completely abolished the increase in respiration due to succinate.

DISCUSSION

Jennings (1904) has described the reactions of Protozoa to electric current and studied the polarizing effect on the cilia of the infusorian body. However, the cathodic migration of *Paramecium* has not previously been used with a view to concentrating the animals for metabolic studies. In the method described here, this migration is supplemented by the negative geotropism of *Paramecium*, a property which Glaser & Coria (1930) have utilized in the preparation of bacteria-free animals. The animals in our suspension were not prepared under sterile conditions, or considered sterile, for this was not necessary in view of the fact that our bacterial blanks, which consisted of determinations of the oxygen consumption of suspensions of animals killed by thermal treatment for 5 min. at 45° C., and of the last wash fluid (the supernatant from centrifugation), gave insignificant values. It seems probable that the reversal of ciliary beat caused by the electric stimulus might serve to dislodge the adherent bacteria during migration through the column of wash

fluid. It is also suggested, in view of the difference in speed of migration of young and old cultures, the increasing number of animals which fail to migrate as the culture ages, and the absence of dividing forms from the final suspension, that some degree of biological segregation occurs during the washing. This is, indeed, desirable, since the physiological state of the organism is known to affect the respiration rate significantly (Boell & Woodruff, 1941; Hutchens, 1939).

Little information is available on the effect of pH on protozoan respiration, but the wide pH tolerance of some types in culture has long been noted. Phelps (1931) found the division rate of *P. aurelia* unaffected over the range 5·9–7·7. Loefer (1938) showed that the limits of growth for *P. bursaria* were 4·0–8·0 with an optimum at 6·8. A similarly wide pH tolerance is reported (Loefer, 1935) for the growth of *Chilomonas paramecium* and *Chlorogonium elongatum*. Mast (1931) found that *Amoeba proteus*, in non-nutrient salt solution, withstood pH 3·8–8·3 for 7 days. Our experiments showed that *Paramecium caudatum* survived and divided in Vegemite-Osterhout solution over the range 4·7–8·5.

It seems that a corresponding pH tolerance of respiration occurs in Protozoa. Von Dach (1942), using *Astasia klebsii* in an inorganic medium, found no variation in respiration at pH 4·5, 5·8 and 7·9, although in the presence of acetate, pH 4·5 depressed the increase in respiration which this substance normally caused. On the other hand, Hall (1941) found an optimum for *Colpidium campylum* at pH 5·5, the rate declining rapidly on the acid side but slowly on the alkaline side. Root (1930) found no effect on the respiratory rate of *Paramecium caudatum* with solutions as acid as 4·5. The results reported here indicate a considerable tolerance on the acid side of the optimum though alkaline conditions decidedly inhibit.

The early work giving rise to the supposition that the respiration of Protozoa is peculiar in being largely or entirely cyanide-stable has been regarded more critically in recent years, and the technical faults which gave rise to misapprehension on this point, such as lack of control of cyanide distillation into alkali, the presence of substrates, lack of control experiments and bacterial blanks, have been discussed. Gradually the uncritical acceptance of work such as that of Lund (1918) who, with the Winkler technique, often omitted control experiments and was severely criticized by Hyman (1919), and also of Shoup & Boykin (1931), whose technique and logic are both most questionable, is being withdrawn and workers now talk of the cyanide-stable respiration of Protozoa with more caution. Future investigations will probably elucidate the respiratory mechanisms linked to the cytochromes and cytochrome oxidase.

With improved techniques, recent workers have found decided inhibition of the respiration of *Paramecium* spp., and other Protozoa formerly reported as cyanide-stable. Thus Pace (1945) found 60% inhibition with young *Paramecium* in 0·001 M-cyanide. Boell (1945) reports about 60% inhibition with cyanide and azide when present in a concentration of 0·01 M. Our results show about 60% inhibition of endogenous respiration by this concentration of cyanide, while azide caused only a 40% inhibition. The effects of azide reported by Boell (1946) in a short communication, are not readily understood. In these experiments, azide at pH 6·02

caused 70% inhibition, whereas at 6·59, a stimulation of 138% occurred, this increase being inhibited by cyanide. Thus some accord seems to have been reached on the cyanide sensitivity of this particular genus and also a recognition that the nutritional state of the organism affects the degree of inhibition; e.g. Pace (1945) states that the percentage inhibition depends on the saturation of the enzyme systems with substrate. This conclusion corresponds to the work of Commoner, on yeast (1939), who reached the same conclusion, the oxygen uptake of starved yeast showing a higher degree of cyanide stability.

There are, too, more precise indications that the cytochrome-cytochrome oxidase system functions in *Paramecium*. The enzyme cytochrome oxidase has been demonstrated quite conclusively in *P. calkinsii* by Boell (1945), using ascorbic acid as a substrate. Cytochrome has been detected spectroscopically in *P. caudatum* by Sato & Tamiya (1937); these workers demonstrated that cyanide prevented the reoxidation of reduced cytochrome. They also report the presence of haemoglobin, though the significance of this pigment in the animal's economy cannot be assessed.

The nature of the mechanisms supplying electrons to the cytochromes is still obscure; there is no knowledge of flavoprotein or co-enzymes in *Paramecium*, though the synthesis of co-enzyme 1 has been demonstrated in *Chilomonas* (Hutchens, Jandorf & Hastings, 1941). Further, nutritional studies which have shown that riboflavin and nicotinic acid are growth factors (Kidder & Dewey, 1946) might indicate that these compounds were functioning in cell metabolism. Succinic dehydrogenase has been reported (Humphrey & Humphrey, 1947), and this enzyme seems to complete a succinoxidase system similar to that found in other animal tissues; this may be compared with the finding of Leichsenring (1925), that succinic acid increased the oxygen uptake of *Paramecium*.

However, it also seems certain that quite a large part of the endogenous respiration of *Paramecium* is stable to cyanide. Indeed, the respiration of few tissues is entirely inhibited by cyanide, but the nature of this cyanide-stable respiration can be considered only in speculation. It is possible that the tissues are functioning on a so-called 'oxygen-debt' and are continuing to produce carbon dioxide. This would be mirrored by a higher respiratory quotient in the presence of cyanide. There are no such R.Q. studies on *Paramecium*, though Pitts (1932), using *Colpidium campylum*, found an increase in R.Q. from 0·65 to 0·90 in the presence of cyanide. The amount of flavoprotein has often been associated with cyanide-stable respiration in other tissues (Groen & Schuyl, 1938; Commoner, 1940), but in Protozoa there is no evidence on this possibility. It is also suggested that cyanide-stable respiration is associated with non-carbohydrate substrates (Commoner, 1940), and certainly in Protozoa there are indications that protein is a more prominent cell substrate than carbohydrate. Thus Leichsenring (1925) showed that protein and amino-acid substrates always increased *Paramecium* respiration more than carbohydrates. Emery (1928) found that the rate of ammonia production by *Paramecium* was proportional to the rate of utilization of some twelve amino-acids. Specht (1935) also reports ammonia production during the endogenous respiration of *Spirostomum*, while, recently, Boell (1946) has claimed that 75% of the respiration of *Paramecium*

calkinsii is due to the use of protein as a substrate; here, too, ammonia production was observed. It is tempting, though hardly justified, to link the protein respiration with the cyanide stability of the flavoprotein D-amino-acid oxidase; but with the scant information available and our ignorance of a possible physiological function of this enzyme, the question must certainly be left open.

SUMMARY

1. A method is described for reducing the numbers of bacteria in a suspension of *Paramecium caudatum* by an electrically directed migration through a sterile column of liquid. The resulting suspension was suitable for metabolic experiments.

2. Details are given of a Cartesian diver respirometer of 'macro' dimensions; this apparatus has a precision of about 10%.

3. The effect of pH on the endogenous respiration of a homogenate of *P. caudatum* showed an optimum in the region 7·0–7·3, with a wide tolerance on the acid side of the optimum but low tolerance on the alkaline side.

4. The endogenous oxygen consumption had a value of 1·9 μl. per 10^4 animals per hr. and was inhibited 60% by 0·01 M-cyanide and 40% by 0·01 M-azide. Methylene blue did not increase the endogenous oxygen uptake.

5. Succinic acid doubled the oxygen consumption, this increase being inhibited by malonate. Methylene blue increased oxygen consumption in the presence of succinate still further, and also abolished the inhibition of this extra respiration by cyanide and azide.

6. It is concluded that *P. caudatum* resembles other animal tissue in possessing an active succinic dehydrogenase.

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THE ABSORPTION OF VOLATILE FATTY ACIDS FROM THE RUMEN

II. THE INFLUENCE OF pH ON ABSORPTION

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(With One Text-figure)

In a previous paper the relative rates of absorption of acetic acid and propionic acid through the rumen wall were discussed (Gray, 1947*a*). While the manuscript was being prepared, Danielli, Hitchcock, Marshall & Phillipson (1945) described a series of experiments from which it was evident that the lower homologues of the fatty acids are absorbed through the rumen wall at different rates when the rumen contents are acid, and that the relative velocities of absorption fall in the series butyric > propionic > acetic. The observations made in this laboratory (Gray, 1947*a*) independently and by different methods were in agreement with these conclusions. There is therefore no reason to doubt that the higher homologues are absorbed preferentially when the rumen contents are acid.

Danielli and his colleagues (Danielli *et al.* 1945), however, concluded that the relative rates of absorption were reversed when the medium was slightly alkaline, and experimental data were presented which, it was claimed, proved that the relative velocities of absorption from a mixture of fatty acids at pH 7·5 fell in the series acetic > propionic > butyric. These latter experimental data were unconvincing. The experiments conducted in this laboratory which are described here lend no support to the claim, or to the general theory in which Danielli and his colleagues suggested that diffusion from acid solutions may proceed through both the intercellular substance and the limiting membranes of the cells themselves, and that only the former pathway is traversed when the solution is slightly alkaline. There is no satisfactory evidence to support the contention that fatty acids diffuse through the rumen wall from a slightly alkaline medium.

METHODS

As in the earlier studies (Gray, 1947*a*), pectin was used as a marker to indicate changes in composition of the aqueous solutions of fatty acids introduced into the rumen. In the course of these experiments it has been proved that pectin does not pass through the rumen wall and that it is not modified when it is introduced in solution into the empty washed rumen.

The concentrations of the fatty acids were estimated by methods (Gray, 1947*b*) which, for the amounts used in these experiments, seem to be more precise than those based on partition chromatography.

For the most part the marker-ratio technique was employed with sheep in which permanent rumen fistulae had been established previously. This allowed observations to be made on the essentially intact rumen in its physiological state. The procedure first used by Barcroft, McAnally & Phillipson (1944) and adopted by Danielli *et al.* (1945) was, however, repeated in some experiments. In these the rumen and its associated organs were isolated from the rest of the alimentary tract by tying off the abomasum just below the omasum, taking care to exclude the epiploic vessels. The oesophagus was occluded in the cervical region. The rumen in this state will be referred to as the 'isolated' rumen.

EXPERIMENTAL

(1) *Changes in the composition of solutions of fatty acids introduced into the rumen at different reactions*

Four experiments were conducted to observe changes in the composition of solutions containing a mixture of sodium acetate and sodium propionate at pH 6·5 and at 8·5 when introduced into an empty, 'intact' rumen. Phosphate was introduced in one solution at each reaction, for there was some suggestion from earlier experiments that the rates of absorption might be influenced by its presence.

The procedure for emptying and washing out the rumen and the methods for the estimation of the fatty acids were those employed previously (Gray, 1947*a*). Between 3 and 4 l. of solution were introduced into the empty, washed rumen. Samples were withdrawn immediately and after 6 hr.

From the results set out in Table 1 it is clear that there were significant changes in the composition of solutions introduced at acid reactions (pH 6·5), indicating, as in previous experiments, the preferential absorption of propionic acid. There was, however, *no evidence of preferential absorption from the alkaline solutions* (pH 8·5), since there were no changes in the proportions of the two acids. Nevertheless, these findings do not preclude the absorption of fatty acids from the alkaline medium, for there would be no such changes if the rates of absorption of the two acids were proportional to their concentrations.

Experiments to test this point were undertaken. In these, the absolute amounts of the acids absorbed under acid and alkaline conditions were determined.

(2) *Measurements of the absorption of acids from the rumen*

If fatty acids are absorbed from an alkaline medium, the fact could be demonstrated by changes in the concentrations of the acids in relation to the concentration of an unabsorbed marker (Gray, 1947*a*). But to measure the actual amounts of each acid which pass through the rumen wall, further information is required. The method used previously to determine the relative rates of absorption of two acids involved the addition of pectin to the solution to act as marker, the assumption being made that pectin could not be absorbed through the rumen wall. It was pointed out then that the absolute amounts of the acids absorbed could be determined only if estimations were made of the quantities of each acid which passed into the abomasum during the experimental period, and of the amounts of each which remained in the rumen at the end of it.

In consequence, experiments were carried out (*a*) to determine whether pectin is or is not absorbed through the rumen wall, and (*b*) to measure the absorption of acetic and propionic acids from solutions of different reactions when introduced into the rumen of an unanaesthetized sheep in a normal physiological state.

Table 1. *Changes in the composition of fatty acid mixtures introduced into the rumen*

Trial no.	pH at start	Time (hr.)	Composition of mixture: Total acid (ml. N acid/100 ml.)	Composition of mixture: [Acetic acid] (%)	Composition of mixture: [Propionic acid] (%)	Phosphate
1	6·5	0	22	44	56	0·25 g. NaH_2PO_4 per 100 ml.
		6	8·6	67	33	
2	8·5	0	30	52	48	
		6	13	53	47	
3	6·5	0	16	57	43	Phosphate absent
		6	5·0	76	24	
4	8·5	0	28	50	50	
		6	9·8	52	48	

Table 2. *Recovery of pectin from the 'isolated' rumen*

	Volume (ml.)	Pectin concentration (g. Ca pectate/100 ml.)	Total pectin (g. Ca pectate)
Introduced	2700	0·29	7·8
Recovered in contents and washings	4000	0·19	7·6

(*a*) *The validity of the use of pectin as a marker for the measurement of absorption of fatty acids from the rumen*

A sheep which had been starved for 3 days was anaesthetized with nembutal and the rumen was isolated according to the procedure described by Barcroft *et al.* (1944). A canula was tied into the rumen, the contents siphoned out and the organs thoroughly washed with several lots of warm water. A solution of pectin was introduced into the empty, washed rumen and the animal was maintained under light nembutal anaesthesia for a further period of $4\frac{1}{2}$ hr. The whole contents were then very carefully collected and the organs washed clean, particular attention being paid to the reticulum and omasum.

Pectin was determined in the original and residual solutions by the method of Nanji & Norman (1928). From the data shown in Table 2, which indicate a recovery of 97% of the pectin introduced into the rumen, it is evident that little or no pectin was absorbed. It may be claimed therefore that pectin is a suitable marker for reference when measuring the absorption of other compounds from the rumen.

(*b*) *The absorption of acetic and propionic acids from the rumen*

Five trials were carried out to determine the absolute amounts of acetic and propionic acids absorbed at different pH levels, both in the presence and in the absence of inorganic phosphate.

Trials 5–8. Mixtures of acetic acid, propionic acid, and pectin, with or without added phosphate, were introduced into the 'intact', empty rumen in distinctly alkaline and acid solutions (pH 10·6 and pH 4·9). In each trial a 400 ml. sample was withdrawn after 2–3 hr. and a 500 ml. sample after 6 hr. The remaining contents of the rumen, together with a small amount of water used to complete the removal, were then collected. Throughout each of these trials the pH of the rumen contents was determined with a glass electrode in samples withdrawn by syringe at half-hourly intervals.

Trial 9. A mixture of acetic acid, propionic acid and pectin at pH 7·5 was introduced into the rumen. A single sample was withdrawn after 5 hr. and the residual contents collected immediately afterwards. The pH of the contents was measured at the end of the experimental period.

In all five trials determinations of the acids and of pectin were made on the original solutions used, and on each sample. The pectin concentrations in the residues were also measured. The analytical data are set out in Table 3 and from these the amounts of each acid absorbed from the rumen and the amounts that passed on to the abomasum may be assessed. The calculations are shown below in full for trial 5; those for the other trials follow the same procedure.

In these calculations the assumption is made that the amount of each acid passing into the abomasum during the experimental period can be estimated from a knowledge of the average concentrations of the constituents in the rumen, and of the amount of pectin lost from the rumen. This is equivalent to assuming that the passage of material from the rumen to the abomasum takes place at regular intervals. The calculations for trial 5 are as follows:

	Acetic acid (ml. N acid)	Propionic acid (ml. N acid)	Pectin (g. Ca pectate)
At 0 hr.	503	526	4·83
In 2½ hr. sample	40·0	27·6	0·488
In 6 hr. sample	42·3	22·7	0·490
In residue	—	—	2·52
∴ Present in rumen at 6 hr. (i.e. before 6 hr. sample taken)	260	139	3·01
Pectin lost from rumen	—	—	1·33
Constituents passed to abomasum (calculated from average rumen composition)	129	113	1·33

Thus the partition of the acids was as follows:

	To first sample	To abomasum	Remaining in rumen at 6 hr.	Total
ml. N acetic acid	40·0	129	260	429
ml. N propionic acid	27·6	113	139	280

Therefore

Acetic acid absorbed = 74 ml. N acid ≡ 15 % of the original acetic acid.
Propionic acid absorbed = 246 ml. N acid ≡ 47 % of the original propionic acid.

Table 3. *Composition of mixtures introduced into the rumen*

Trial no.	pH at start	Volume introduced (ml.)	Time hr.	Constituents of rumen contents				Residue (incl. washings)		Phosphate
				Total acid (ml. N acid/ 100 ml.)	[Acetic acid] (%)	[Propionic acid] (%)	Pectin (g. Ca pectate/ 100 ml.)	Volume (ml.)	Pectin (g. Ca pectate/ 100 ml.)	
5	4·9	3500	0	29·4	49·0	51·0	0·138	—	—	Absent
			2½	16·9	59·2	40·8	0·122	—	—	
			6	13·0	65·1	34·9	0·098	3700	0·068	
6	10·6	3700	0	27·3	48·3	51·7	0·449	—	—	
			3	23·6	48·9	51·1	0·388	—	—	
			6	20·5	47·8	52·2	0·341	3600	0·222	
7	4·9	3700	0	27·1	49·0	51·0	0·454	—	—	0·25 g. NaH_2PO_4 per 100 ml.
			2	17·6	56·3	43·7	0·360	—	—	
			6	11·6	65·5	34·5	0·299	4700	0·224	
8	10·6	4000	0	30·5	45·6	54·4	0·442	—	—	
			2	27·0	46·8	53·2	0·422	—	—	
			6	20·4	48·6	51·4	0·302	4000	0·271	
9	7·5	4000	0	24·8	52·3	47·7	0·267	—	—	Absent
			5*	16·6	53·9	46·1	0·178	4720	0·150	

* 5 hr. sample = 485 ml.

Table 4. *The fate of fatty acids introduced into the rumen*

Trial no.	pH at start	ml. N acid										Phosphate
		Introduced into rumen		Removed in sampling		Passed to abomasum		Remaining in rumen after 6 hr.		Absorbed from rumen		
		Acetic	Propionic	Acetic	Propionic	Acetic	Propionic	Acetic	Propionic	Acetic	Propionic	
5	4·9	500	530	40	28	130	110	260	140	70	250	Absent
6	10·6	490	520	46	48	160	170	280	300	4	2	
7	4·9	490	510	40	31	94	79	300	160	56	240	0·25 g. NaH_2PO_4 per 100 ml.
8	10·6	560	660	50	58	120	140	400	430	−10	32	
9	7·5	520	470	—	—	140	120	400*	340*	−20	10	Absent

* Remaining in rumen at 5 hr.

An extra significant figure has been retained in the data and throughout the calculations in order to minimize the accumulation of arithmetical errors. Results for all five trials are summarized in Table 4.

Changes in the reaction of the rumen contents during the first four of these trials are plotted in Fig. 1. In trial 9 the reaction was not significantly altered (pH 7·4) at the end of the experimental period, and may be considered to have remained constant throughout the trial.

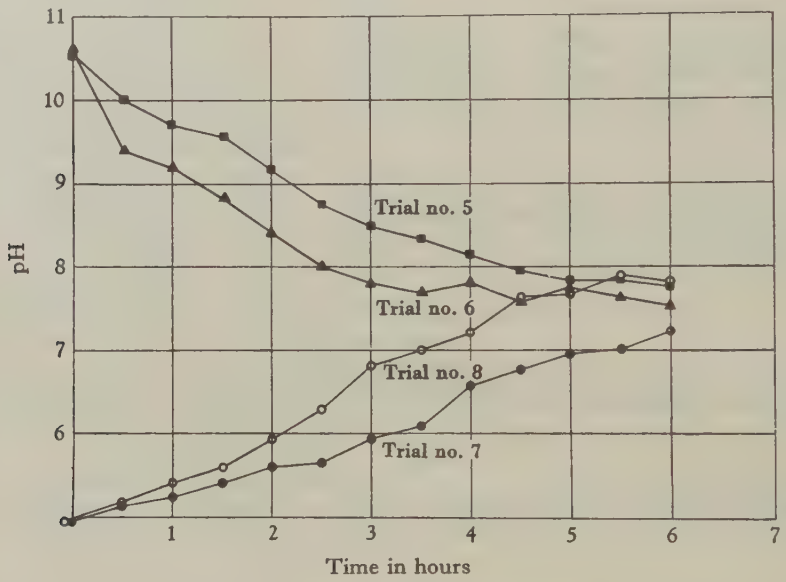

Fig. 1. pH of rumen contents during trials 5–8.

It may be concluded from trials 5 and 7 that while the reaction of the solution in the rumen was changing from about pH 5 to 7, absorption of both acids took place rapidly. The presence of inorganic phosphate had no significant effect on the total quantities of the acids absorbed, and it may therefore be concluded that the apparent effect of phosphate noticed in the earlier experiments (Gray, 1947*a*) was, in fact, an effect due to the different pH levels of the solutions used in those trials where phosphate was included.

In trials 6 and 8 little or no absorption of acid took place while the reaction of the solution in the rumen was changing from about pH 10·5 to 7, and in trial 9 no significant absorption took place while the reaction of the solution in the rumen remained practically constant at pH 7·5.

(3) *The absorption of acetic acid from the 'isolated' rumen at alkaline reactions, as found by direct and by indirect methods*

As the claim that the fatty acids are absorbed through the rumen wall at pH 7·5 was not supported by the evidence in the trials just described, it was decided to check the results obtained when using the indirect method of Danielli *et al.* by a direct measurement of the recovery of acetic acid introduced into an 'isolated'

rumen. Both methods were employed at the same time in each of two trials so that the experimental conditions were identical for each procedure. Acetic acid was used alone since, according to Danielli *et al.*, it is absorbed more rapidly than its higher homologues from an alkaline solution.

The operative procedure for isolating the rumen from the rest of the alimentary tract has already been referred to. In these trials solutions of sodium acetate at reactions between pH 7·5 and 8·0 were introduced into the rumen for periods of 3 hr., after which samples were withdrawn for analysis. In one trial sodium acetate solution was then added to the rumen contents; in the other acetic acid was used

Table 5. *Recovery of acetic acid from the 'isolated' rumen*

Time		Trial no. 10	Trial no. 11
	Solution introduced		
0 hr.	Volume (ml.)	2810	2980
	ml. N acid	497	527
	ml. N acid/100 ml.	17·7	17·7
	First sample taken		
3 hr.	Volume (ml.)	158	165
	ml. N acid	19·0	22·3
	ml. N acid/100 ml.	12·0	13·5
	Solution added		
3 hr. 1 min.	Volume (ml.)	497	100
	ml. N acid	88·0	106
	ml. N acid/100 ml.	17·7	106
	Second sample taken		
3 hr. 6 min.	Volume (ml.)	175	153
	ml. N acid	22·2	24·0
	ml. N acid/100 ml.	12·7	15·7
	Residues and washings		
3 hr. 7 min.	Volume (ml.)	4600	5500
	ml. N acid	520	561
	ml. N acid/100 ml.	11·3	10·2

instead. In each case 5 min. were allowed for mixing, and this was assisted by massaging the abdomen of the animal. Then another sample was withdrawn so that the change in acid concentration could be measured. Immediately afterwards, the whole contents of the isolated organs, together with washings used to complete their removal, were collected.

The original solutions, the residues, and the two intermediate samples were steam-distilled according to the usual procedure and the distillate titrated with standard alkali to determine the concentrations of acid present. Data from these trials are given in Table 5.

The loss of acetic acid from the rumen is calculated directly from these measurements and also indirectly by the procedure used by Danielli *et al.*

Loss of acetic acid from the 'isolated' rumen

(1) *By direct measurement*

Trial	Introduced ml. N acid	Recovered ml. N acid	Loss (%)
10	585	561	4
11	633	607	4

(2) *By indirect measurement* (procedure of Danielli *et al.*)

If V = volume of rumen contents at 3 hr., then for trial 10

$$\frac{(V+497-158)\times 12{\cdot}7}{100}-\frac{V\times 12{\cdot}0}{100}=88-19.$$

$V=3700$ ml.

Acid introduced at 0 hr. = 497 ml. N acetic acid
Acid present at 3 hr. = 444 ml. N acetic acid
Loss = 53 ml. = 11 %

And for trial 11:

$$\frac{(V+100-165)\times 15{\cdot}7}{100}-\frac{V\times 13{\cdot}5}{100}=106-22{\cdot}3.$$

$V=4270$ ml.

Acid introduced at 0 hr. = 527 ml. N acid
Acid present at 3 hr. = 576 ml. N acid
Gain = 49 ml. = 9 %

Three significant figures have been quoted throughout the data from these experiments, and used in the calculations from them, but no claim is made for the order of accuracy that this implies. The amount of acetic acid computed from the direct measurement probably involves an error of the order of 1 %; but the result from the indirect method is undoubtedly less accurate. Further reference is made to this point in the discussion.

DISCUSSION

The experiments that have been described here prove that, while there is rapid absorption of volatile acids from solutions introduced into the rumen at acid reactions, there is little or no absorption when the reaction of the rumen contents is alkaline.

This latter finding is in conflict with the conclusion of Danielli *et al.* (1945). The direct measurement of the absorption of acetic acid from an 'isolated' rumen, carried out at the same time as the indirect measurement employed by these workers, has shown that their procedure may give rise to misleading results. One reason for this inaccuracy may be inherent, not in the method itself, but in certain limitations which were arbitrarily imposed in its application. The accuracy of the calculation of the volume of the rumen contents at the end of the experimental period depends upon the accuracy with which the *difference* in concentrations of acid can be measured before and after the addition of a known amount of acid. A small error in the estimation of either of these concentrations will lead to a very much larger error in the computed difference between them so long as that difference is small in relation to the original and final concentrations. This larger error will then be reflected in the volume calculated, and therefore in the estimation of the acid present at the end of the experimental period. In the three experiments described by Danielli *et al.*, the proportions of acid absorbed in 2 hr. were claimed to be approximately 30, 7 and 16 %. The change in acid concentration at the end of the first of the experiments, in which the most significant amount of absorption was calculated, was from 19·7 to 21·8* ml. N acid/100 ml. If these measurements were

* The figures quoted by Danielli *et al.* are 19·7 and 21·0. The latter is thought to be a misprint, and the use of 21·8 instead gives approximately the volume calculated by the authors. A number of other figures in the same table appear to be inconsistent among themselves, but the above argument remains unaffected.

each subject to an error of only ±0·5%, then the amount of acid calculated to remain in the rumen at the end of the experimental period would have been subject to an error of approximately ±10%. The smaller absorption (7%) claimed in their second experiment, in which a much greater change was brought about in the acid concentration, lends support to the view that this source of error may have complicated the interpretation of their first experiment. The acid concentrations were determined in 2 ml. samples by the method of McAnally (1944), the accuracy of which was not stated.

A second possible source of error lies in the fact that the acid added at the end of the experimental period must be completely mixed with the whole contents of the reticulum and omasum, as well as with that of the rumen; the former two organs are not always in free communication with the rumen, and while massage of the abdomen will promote a more rapid mixing of the contents, some uncertainty of its completeness remains. If mixing between these organs were incomplete, the estimation of the volume by this means would tend to give low results and the amount of absorption calculated from this would therefore be too great.

The exact separation of the abomasum from the omasum in the isolation of the rumen is not easily achieved. In some cases during the present work it was found that a small pocket of the abomasum had been left above the ligature. If such a pocket were extensive enough, it might offer an avenue for the absorption of significant amounts of acid which would therefore be wrongly attributed to passage of the acid through the rumen wall.

It was claimed by Danielli *et al.* that not only were the fatty acids absorbed in the alkaline range, but also that the order of the rates of absorption was the reverse of that obtaining under acid conditions. Their data, however, show only very small changes in the composition of three-acid mixtures placed in the rumen and these do not all indicate the order of absorption claimed. The partition chromatographic method of Elsden (1946) was used for these analyses, and for this Elsden quotes figures showing a standard deviation of between 2 and 3% from the mean recovery in each of the three acids determined in such a mixture. The changes in the proportions of the acids which were present in the alkaline mixtures used by Danielli *et al.* do not therefore appear to be significant.

Two minor points of interest arise incidentally out of the present trials. In the first place a rapid change of reaction took place in the rumen when a solution was introduced at pH 5 or at 10. Both solutions were brought approximately to neutrality within 3 hr. when no inorganic phosphate had been included, and within about 5 hr. when phosphate was present. Danielli *et al.* (1945) have pointed out that the change from acid to neutral reaction must, in part, be brought about by the passage of free acid through the rumen wall. In the older view the change would have been ascribed entirely to the advent of alkaline saliva into the rumen. The second point to be noted is that, while it has been claimed that only small quantities of fatty acids are to be found in the abomasum (Phillipson & McAnally, 1942), and that significant quantities of such acids do not appear in the blood draining the abomasum (Barcroft *et al.* 1944), yet the trials described in part (2) (*b*) demonstrate

the passage of considerable amounts of acid into the abomasum under the conditions in which the experiments were made.

SUMMARY

1. Mixtures of acetic and propionic acids introduced into the rumen at pH 8·5 showed no significant changes in the relative proportions of the acids in a period of 6 hr.

2. Pectin was shown to remain unchanged and unabsorbed in the 'isolated' rumen. By using it as a marker the course taken by fatty acids introduced into the empty rumen was followed. The acids found their way from the rumen, in part by absorption through the rumen wall and in part by passing on to the abomasum.

3. Absorption of both acetic and propionic acids took place readily at acid reactions. Between 10 and 14% of the acetic acid and about 47% of the propionic acid introduced was absorbed within 6 hr. The amounts absorbed were not altered by the inclusion of inorganic phosphate in the mixture.

Absorption did not occur to any significant extent from similar solutions introduced at reactions $\geqslant$ pH 7·5.

4. In two experiments acetic acid was not absorbed from an 'isolated' rumen when introduced as a slightly alkaline (about pH 7·5) solution of sodium acetate. Recovery after 3 hr. was measured directly and found to be 96%. In the same experiments the indirect method of Danielli *et al.* (1945) yielded varying results (89 and 109% recovery).

5. It is concluded that while rapid absorption of fatty acids from the rumen takes place at acid reactions, there is no absorption at all from alkaline solutions.

The author is greatly indebted to Mr I. G. Jarrett who carried out all the necessary operative procedures, and to Mr H. R. Marston, Chief of the Division, for his continued interest and advice.

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THE SENSORY PHYSIOLOGY OF THE SHEEP TICK, *IXODES RICINUS* L.

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(With Thirty-one Text-figures)

The sensory perceptions of several species of ticks, notably *Argas persicus* (Hindle & Merriman, 1912), *Boophilus annulatus* and *Rhipicephalus sanguineus* (Krijgsman, 1937), *Ixodes ricinus* (Totze, 1933) and *I. persulcatus* (Mironov, 1939), have been studied systematically in the laboratory. There are, in addition, many scattered references in the literature to the behaviour of ticks and some incomplete descriptions of their sense organs. The chief aim of the present work has been to investigate the orienting reactions of the sheep tick, and the role of the special senses, in relation particularly to those stimuli the tick will encounter in its natural environment. With such an object in view, a knowledge of the ecology and behaviour is clearly a desirable, if not an essential, starting-point. There was little understanding of these subjects at the time Totze was writing and, in consequence, there was a tendency for the laboratory observations to lack meaning or to acquire a distorted significance. A fresh examination of the sensory physiology is justified by the increased knowledge of the ecology of the sheep tick in Britain, a subject which has been studied intensively from 1932 onwards. As a preliminary to the present work some weeks were spent observing the behaviour of a population of active ticks under natural conditions. These observations proved of value both by suggesting lines of investigation and, finally, in attempting an interpretation of the significance of the reactions observed in the laboratory.

The life history, distribution and environment of the sheep tick in Britain are fully described in the papers of MacLeod (1932, 1936) and Milne (1944, 1945, 1948*b*). *Ixodes* is most abundant on rough hill or moorland grazings where there is thick vegetative cover. A small proportion of the total tick population is carried by the wild fauna, but far larger numbers are fed by domestic stock, with the hill sheep as the outstanding host (Milne, 1948*a*). Each of the three stages, larva, nymph and adult, seeks a blood meal by climbing the stems of grasses, rushes or the like and waiting there for a host to pass by. After engorging, the ticks drop off and find shelter in the deeper layers of the vegetation. Most of the life of the tick is spent on the ground. Under normal circumstances the life cycle requires 3 years for completion (Campbell, 1946), whereas the usual engorgement periods of the larva, nymph and female are only 3–4, 4–5 and 7–12 days respectively.

POSTURE AND MOVEMENTS

The unfed tick may adopt one of two characteristic attitudes when at rest. In the 'questing' posture (the 'waiting attitude' of Mironov, 1939), assumed when the tick is on the alert, the forelegs are extended rigidly to the front in the manner shown in Fig. 1 A. Sometimes they are held out immobile thus giving the tick an air of tense expectancy; sometimes they are waved actively in the manner of antennae. The forelegs, which bear Haller's organ and other sensilla have, indeed, often been compared in their function and carriage with the antennae of insects. A hungry

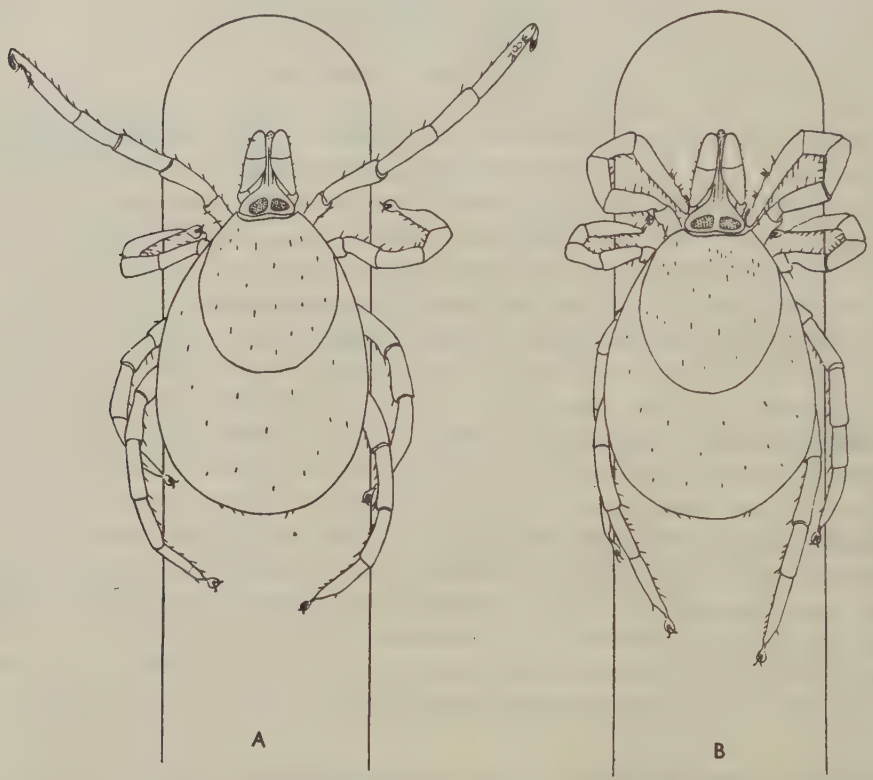

Fig. 1. Unfed female ticks at rest near the tip of a glass rod. A, questing attitude; B, resting posture.

tick spends some time in questing even in the absence of sensory stimulation, although an attitude of repose with the forelegs folded (Fig. 1 B) is more usually adopted when the tick is entirely undisturbed by external influences. This posture may be maintained for weeks or months on end.

When walking, the hungry tick holds the forelegs slightly raised above the general level of the body and waves them backwards and forwards in a regular manner as it progresses. The presence of tarsal pulvilli permits climbing on any surface, however smooth. After a recent moult the tick is not hungry and tends to make use of the forelegs in walking; the same is true of older ticks which have been disturbed

roughly and are running rather than walking in their usual deliberate manner. Ticks walking in this way seldom respond to otherwise favourable stimuli. The hexapod larvae also quest with the forelegs when at rest. They cannot, however, both quest and progress at the same time and so are forced to make intermittent use of the forelegs in walking.

A hungry tick in repose quests instantly in response to certain forms of stimulation. This postural response can be used in investigating the sensory perceptions.

REACTION TO GRAVITY

Hindle & Merriman (1912) concluded that *Argas persicus* showed no well-defined reaction to gravity. Three species of Ixodidae, all of which are known to climb vegetation before transferring themselves to a passing host, have given variable results. MacLeod (1935), using large numbers of larvae of *Ixodes ricinus* in a vertical glass tube, recorded aggregations at the base when the temperature was below 12 or above 30° C., whereas the larvae collected mainly at the top of the tube at intermediate temperatures. He concluded that negative geotaxis operated between 14 and 24° C., and positive geotaxis at temperatures outside this range. Mironov (1939) contends that the tick *I. persulcatus* is negatively geotactic; he describes how in a uniform humidity the unfed ticks climbed to the top of paper and glass models of plants and remained there in a waiting attitude, usually resting on the lower surface of the 'leaves'. Krijgsman (1937), on the other hand, found that the larvae of *Boophilus annulatus* in a vertical glass tube assumed a random distribution and were apparently unaffected by gravity.

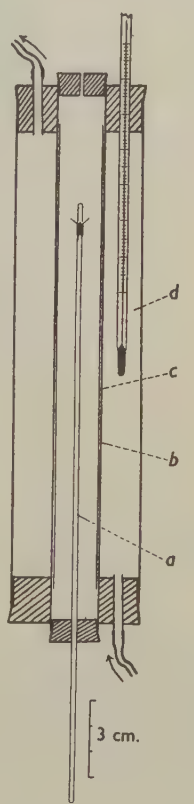

Fig. 2. Arrangement for testing the gravity and humidity responses of single ticks. *a*, glass rod on which the ticks climb; *b*, inner glass tube; *c*, filter paper; *d*, outer water jacket.

Method

The response to gravity has been reinvestigated by observing the individual behaviour of unfed ticks walking on lengths of glass rod. The standard rod, 0·2 cm. in diameter and 24 cm. in length, was marked at intervals of 1 cm. with rings of cellulose paint. These rods proved to be most satisfactory models of the stems of rushes or grasses, which in the natural environment are favoured sites for climbing. In earlier experiments the humidity was controlled by housing the rod in a glass tube which could be partly lined with filter paper moistened with the appropriate saturated salt solutions (Fig. 2). The temperature was varied or maintained constant by drawing water at the required temperature through an outer water jacket. The rod, passing loosely through two

corks closing the ends of the tube, could be slipped from either so that the path of the tick round the ends was not interrupted. In later experiments humidity was not controlled and the rods were simply inserted in large corks. When the tick approached the fixed end this was removed and the other end inserted without changing the inclination of the rod. With careful handling the movements are not affected; when the tracks are confined to one end, the rod itself need not, of course, be touched. The ticks are induced to climb on to the rod by slightly warming it. Unfed nymphs were generally used, as adults often become agitated after handling and fall off after progressing a short distance.

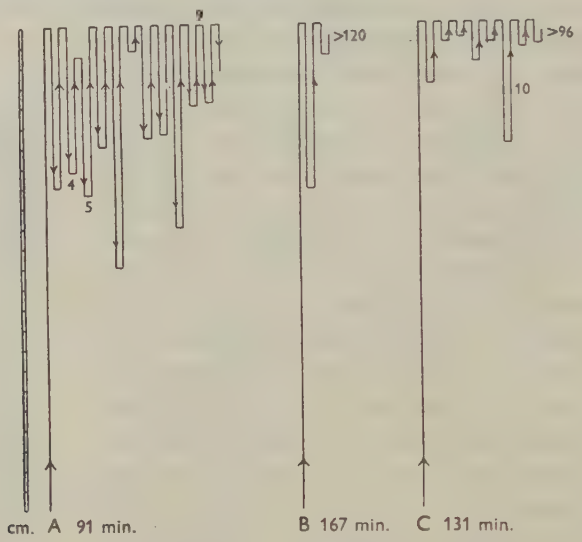

Fig. 3. Movements of three unfed nymphs on a vertical 24 cm. rod at 20° C. The figures by the rod indicate the time in minutes spent at rest at any particular point, and those at the base the duration of the experiment. The tracks (which are in reality superimposed) are shown as if displaced laterally.

Results

Some typical examples of the movements of ticks on vertical glass rods at 20° C. and 86% R.H. are shown in Fig. 3. If the tick is introduced at the base of the rod, it climbs steadily to the tip, questing as it goes. On reaching the tip it swiftly touches the end of the rod with the forelegs, then walks round the tip with the forelegs waving. An excited tick may 'rear up' freeing the second and even the third pair of legs from contact with the substratum. After a short while it begins to descend. On progressing downwards for a variable distance, however, the regular alternating movements of the forelegs are interrupted and, turning round, the tick again walks to the tip of the rod. These movements are often repeated several times before the tick finally comes to rest near the tip, questing at first but later folding its legs and questing intermittently.

The number of consecutive descents, turns and ascents are very variable, as are the distances of successive descents from the tip. In the examples given in Fig. 3, one nymph turned upwards no less than eleven times, another eight times and

another twice only. Yet, after experience of the tip, there was only one downward turn during upward climbing. The final position on the rod is also very characteristic, the tick often settling down a few millimetres below the tip with the capitulum uppermost. Less frequently the final position of rest is farther down the rod. The body may sometimes be inverted, but the longitudinal axis invariably lies in the vertical plane.

Some twenty tracks obtained at 20° C. showed the same characteristic pattern. This behaviour, which clearly tends to bring the ticks to the tip of the rod and to keep them there, is thus accomplished by a succession of turning reactions during the descent from the tip, followed by a kinetic response. But whether this is a response to gravity or merely a sequence of reactions set in train by arrival at the

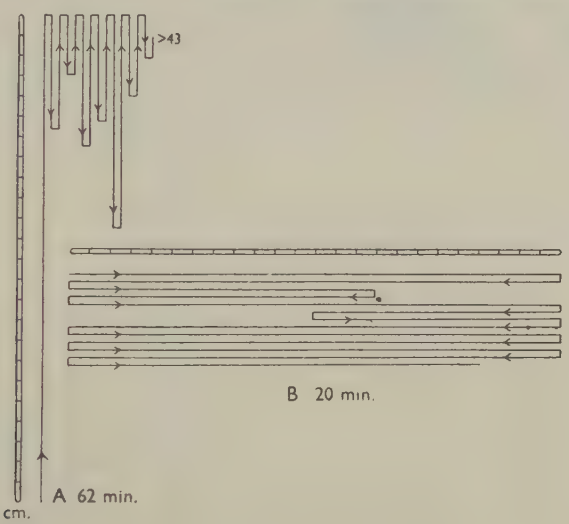

Fig. 4 A, B. Tracks of the same unfed nymph climbing on vertical and horizontal rods.

tip of the rod is uncertain. An answer to this question was sought by following the movements in the following situations:

(*a*) When ticks are allowed to walk along a horizontal rod, turning movements, which are never pronounced, do not appear to occur with greater frequency near the free ends of the rod. Neither do the ticks tend to come to rest very readily. The tracks of the same nymph climbing on a vertical and on a horizontal rod are shown in Fig. 4 A, B.

(*b*) A curved rod is used. The tip is bent over so that the rod is U-shaped with one long arm, which is held in a vertical position. Ticks introduced at the base of the long arm in an upward climbing position must therefore walk downwards before reaching the actual tip. The movements of two nymphs are shown in Fig. 5. On arrival at the apex of the curved portion they usually behave as though they have reached the tip. The individual shown in Fig. 5 A, after climbing over the top of the U, turned while walking down the further side, climbed over the top again, turned, and finally came to rest on the underside of the rod at the highest point.

The tick shown in Fig. 5 B constantly reversed while climbing downwards without, however, coming to rest.

(*c*) The ticks were allowed to climb vertical glass rods 150 cm. in length. With this length of rod ticks introduced near the base rarely walked to the top. Fifteen nymphs climbed to an average height of only 44 cm., and, as Fig. 6 shows, turning movements are very poorly expressed. Usually the tick climbs to the highest point, turns downwards and walks straight down to the base without turning upwards again. One tick only reached the tip, and then proceeded to execute the characteristic turning movements seen on the 24 cm. rod (Fig. 6 D), finally coming to rest 1 cm. below the tip.

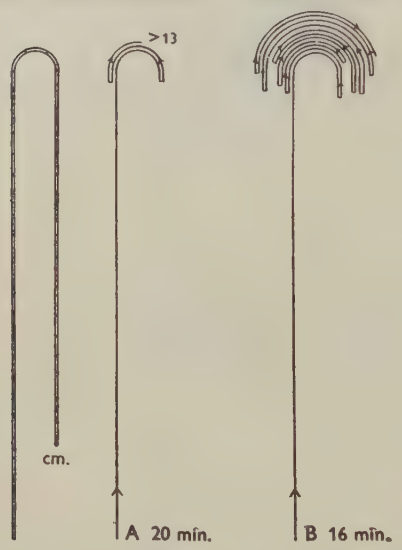

Fig. 5. Tracks followed by two unfed nymphs on curved glass rods.

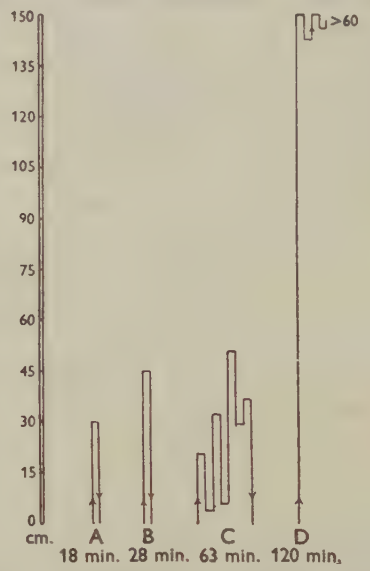

Fig. 6. Tracks of four unfed nymphs climbing vertical rods 150 cm. in length.

(*d*) The distribution of fifty unfed nymphs in a tube 1 cm. in diameter was followed. After introducing the ticks and distributing them as evenly as possible, the tubes were closed by perforated, gauze-covered corks which could be pushed inside the ends so as to make a chamber 24 cm. in length. This was marked into four sections of 6 cm. each. The tubes were either laid lengthwise on the bench or clamped in a vertical position. After 30 min. the number of ticks collecting in each section was counted. Each experiment was repeated ten times at 19° C. and 86% R.H.

The results, which are given in Table 1, showed that although there was a strong tendency for the ticks to aggregate at the ends of the chamber, only a small excess occurred at the top of the vertical tube. The average of 23·2 ticks in the uppermost section and 20·5 in the lowest section did not differ significantly from the numbers collecting at the ends of the horizontal chamber.

The results of these tests are not easy to interpret. The behaviour in situations (*a*) and (*b*) seems to imply that gravity may have some influence on the turning movements, while in (*c*) and (*d*) it seems to have none. Two points are clear, however. First, if there is a slight negative geotaxis, ticks may on occasion walk downwards for considerable distances without turning, as is well shown on the very long vertical rods. The gravity response is not therefore of the more stereotyped kind displayed by certain animals which possess static organs. Secondly, the tendency to turn upwards is greatly enhanced if there is recent past experience of arrival at the free end of some vertical object. This perception is certainly of a tactile nature. Arrival at the summit of a curved rod may perhaps satisfy these sensory requirements, even though the tip has not been reached, while arrival at the end of a closed tube seemingly does not. The random distribution assumed by the ticks in vertical tubes is at variance with the findings of MacLeod (1935), but agrees with the experience of Krijgsman (1937) with *Boophilus* larvae. Nevertheless, this particular observation is of very doubtful significance in interpreting the vertical movements of ticks under natural conditions (see p. 199).

Table 1. *Average distribution of fifty unfed nymphal ticks in vertical and horizontal tubes*

Vertical		Horizontal	
Section	Mean $\pm \sigma_M$	Section	Mean $\pm \sigma_M$
Top 1	23·2 ± 1·6	Left 1	20·3 ± 1·6
2	3·7 ± 0·7	2	4·8 ± 0·6
3	2·6 ± 0·6	3	4·4 ± 0·8
Bottom 4	20·5 ± 1·7	Right 4	20·5 ± 1·6
Total	50·0	—	50·0

Gravity and temperature

In order to bring the present work into line with previous work on *Ixodes ricinus*, some observations were made on ticks climbing vertical 24 cm. rods at different temperatures. The rods were set up in constant temperature rooms running at 7, 11 and 25° C. and unfed nymphs previously kept at 19° C. were used. At least eight tracks, of which several are shown in Fig. 7, were obtained at each temperature.

Characteristic movements, such as have already been described, are expressed with only minor variations at all three temperatures. At 25° C. the ticks were excited and the descents after reaching the tip exaggerated (Fig. 7 E, F). At 7° C. climbing was slow and laborious as this temperature was only about 2° C. above the point at which chill-coma supervened: yet the ticks turned upwards after beginning the descent and finally came to rest near the tip in the usual manner (Fig. 7 A, B). These observations do not then support the view (MacLeod, 1935) that temperature has a differential effect on the vertical movements.

Movements of engorged ticks

Although the unfed tick climbs any object with ease, this is not the case with the engorged stages of *Ixodes ricinus*. This is partly due to the great weight of the ingested blood and partly to the altered set of the legs. Climbing a highly convex object such as a glass rod or a plant stem is difficult for engorged larvae and nymphs which, after progressing for a short way, lose their foothold and fall. The engorged female is incapable of climbing at all. de Meillon (cited by Fraenkel & Gunn, 1940) has pointed out that the dog tick *Rhipicephalus sanguineus* climbs directly upwards on a vertical surface because the centre of gravity of the engorged tick comes to lie behind the legs. This has been confirmed with engorged females of *Ixodes canisuga*.

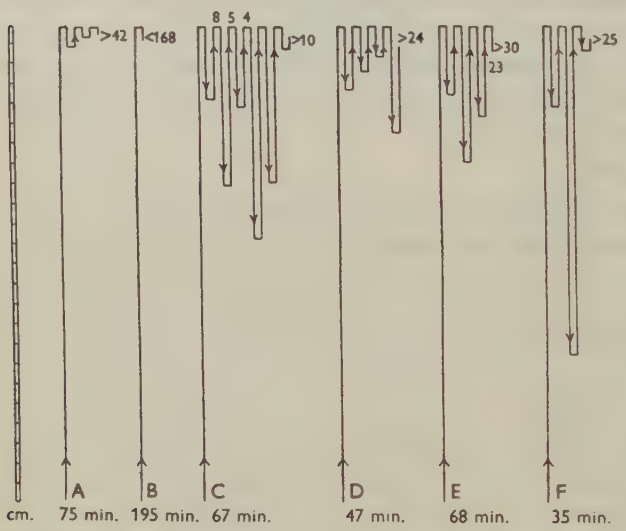

Fig. 7. Vertical movements of six ticks on 24 cm. glass rods at different temperatures. A, B, 7° C.; C, D, 11° C.; E, F, 25° C.

Fig. 8. *Ixodes canisuga*. Path of an engorged female climbing a vertical sheet of paper which was inverted at point *I*. The track is drawn relative to the background.

This species takes a smaller blood meal than *I. ricinus* and the engorged stages are much more agile climbers. On a vertical sheet of paper the ticks walk directly upwards. If the paper is rotated so that the tick is for the moment progressing downwards, the heavy body tends to topple sideways, the legs are dragged round, and the tick resumes its upward course (Fig. 8). This is a gravitational reaction of a gross type, the behaviour resembling that of the louse *Haematopinus* after a full blood meal (Weber, 1929). With *Ixodes canisuga* this response plays a definite role in the behaviour under natural conditions (see p. 203).

RESPONSE TO HUMIDITY

Method

For investigating the general humidity behaviour small alternative chambers of the type described by Wigglesworth (1941) were used. The base of the chamber consisted of a Petri dish divided by a central glass partition into two cavities for

containing the saturated salt solutions. On this rested a platform of voile stretched tightly on a wire ring. The roof of the chamber was a further Petri dish, 9 cm. in diameter, inverted and exactly fitting over the rim of the lower dish. The advantage of this type of chamber was that the humidity could be changed at will, without disturbing the ticks, merely by lifting the ring and replacing the lower dish. The ticks walk on the walls and roof as well as on the voile floor, but carry out normal orienting responses in these situations. Fifty unfed nymphs or twenty-five engorged nymphs could be introduced simultaneously.

The reactions of unfed ticks to humidity were also followed individually as they climbed glass rods. The apparatus has already been described (p. 147, Fig. 2). The rods were held in glass tubes partly lined with strips of filter paper. For exposure to alternative humidities two such strips, moistened with the appropriate solutions and adhering firmly to the inner wall, were set end to end, $\frac{1}{4}$ cm. apart. Saturated salt solutions of the following salts were used, all at 19° C.:

$MgCl_2$, 34% R.H.; $Ca(NO_3)_2$, 44% R.H.; NH_4NO_3, 67% R.H.; NaCl, 78% R.H.; KCl, 86% R.H.; Na-tartrate, 92% R.H.; $Ca(H_2PO_4)_2$, 95% R.H. (Buxton, 1931; Buxton & Mellanby, 1934).

The humidity gradient along the rod was plotted with the aid of a weighing hygrometer in the form of a small scroll of paper 1 cm. long which was slipped over the rod and allowed to slide down to the required point where it came to rest against a plasticine stop. With humidities of 34 and 86% R.H., and with the tube vertical, the steepest part of the gradient was found to be at least 20% R.H./cm. irrespective of whether the dry or the wet side was uppermost. 1·5 cm. from the junction of the strips the humidity was about 40% on the dry, and about 84% on the wet side. Evidently the proximity of the evaporating surfaces to the rod ensures that the humidity gradient is not unduly disturbed by convection currents, in spite of the height of the chamber.

Results

The general humidity behaviour may be illustrated by the results of a typical experiment where fairly extreme alternative humidities (34 and 100% R.H.) were offered (Table 2). Fifty unfed nymphs, taken from saturated air in the culture tubes, were placed in the chamber, the distribution and the numbers of ticks on either side active or at rest being recorded at intervals during a period of 6 days. In the control chamber the ticks were exposed to uniformly saturated air.

After introduction into the chamber nearly every tick is at first intensely active (Table 2). During this phase, particularly during the first few hours, most of the ticks are found on the dry side. For example, during the first hour 165 position records were from the dry side and thirty-five from the wet side. This is due to a simple avoiding reaction: the ticks approach the humidity boundary, turn round and walk back into the dry side. Gradually, as the effects of the disturbance subside, the ticks begin to settle down, questing at first but later folding their legs. Because of the avoiding response most of the ticks settle down on the dry side. Thus after 4 hr. thirty-seven were on the dry and thirteen on the wet side; and of these

thirteen and eight respectively had come to rest. After 24 hr. nearly every tick is inactive at any particular moment of observation. Nevertheless, from the first to the sixth day, ticks gradually leave the dry side and collect in moist air. And after 6 days every tick was on this side. At this juncture the ticks were again disturbed and rendered active by removing the cover of the chamber and redistributing them in the centre of the arena. As Table 2 shows, the original behaviour is now restored; renewed avoidances of moist air are seen and the ticks again tend to come to rest mainly on the dry side. In the control chamber the fifty nymphs are distributed at random, but otherwise show the same general tendency to come to rest after disturbance.

Table 2. *The humidity behaviour. Distribution and activity of fifty unfed nymphs during exposure to alternative low and high humidities*

Duration from start of experiment			Alternative chamber				Control chamber			
			34% R.H.		100% R.H.		100% R.H.		100% R.H.	
Days	Hr.	Min.	Total	No. active	Total	No. active	Total	No. active	Total	No. active
		15	38	38	12	12	29	28	21	21
		30	44	42	6	6	25	24	25	23
		45	42	40	8	7	28	26	22	21
	1	0	41	37	9	7	26	23	24	20
	4	0	37	24	13	5	25	19	25	17
1	0	0	26	1	24	0	22	0	28	0
2	0	0	11	0	39	0	22	0	28	0
3	0	0	5	0	45	0	21	0	29	0
4	0	0	4	0	46	0	22	0	28	0
5	0	0	3	0	47	0	23	0	27	0
6	0	0	0	0	50	0	23	0	27	0
				Disturbed				Disturbed		
6	0	15	38	36	12	11	28	27	22	21
6	0	30	35	32	15	11	30	28	20	17
6	0	45	38	31	12	8	22	17	28	22

The mechanism of humidity reaction

This behaviour suggests that there are two elements in the response to alternative humidities, first, a directed reaction (taxis), which is expressed by avoiding moist air after previous exposure to a saturated atmosphere, and secondly, an undirected reaction (kinesis) which tends to bring the tick to rest in damp air. It appeared probable that the expression of these reactions was dependent on the state of desiccation.

For studying the effect of desiccation on the general humidity behaviour, batches of fifty unfed nymphs, either removed directly from the moist culture tubes or previously desiccated overnight at 50% R.H., 25° C., were offered relative humidities of 95 and 34% in the alternative chamber. After this comparison had been made the desiccated ticks were returned to saturated air for 48 hr. and tested again. The results of such an experiment are given in Table 3.

The normal ticks from the culture tubes behave in the manner previously described, avoiding moist air strongly and collecting at first on the dry side. And

after they have been disturbed and redistributed the response persists. The desiccated ticks, in contrast, fail to show any avoiding reaction, so that during the initial period of activity distribution in the chamber is random. Gradually, however, they begin to collect and come to rest in the moist half of the chamber. And 1 hr. 45 min. later, when the ticks were disturbed, random distribution is soon followed again by aggregation on the moist side. However, after exposure to a saturated atmosphere for 48 hr., the 'desiccated' ticks no longer respond in this way: the avoidance of moist air has returned and the aggregating behaviour in moist air is no longer in evidence. Now it has been previously shown that after desiccation ticks are able to take up water through the cuticle when exposed to humidities higher than about 90% R.H. (Lees, 1946). Clearly then the return of the normal humidity behaviour is associated with the restoration of the water balance.

The following observations have been made with the object of throwing further light on the humidity behaviour.

Table 3. *Distribution of fifty unfed nymphs in an alternative chamber providing low and high humidities*

A, normal ticks from saturated air; B, ticks desiccated for 16 hr. at 50% R.H., 25° C.; C, desiccated ticks subsequently exposed to saturated air for 48 hr. at 25° C.

Duration from start of experiment		A		B		C	
Hr.	Min.	34% R.H.	95% R.H.	34% R.H.	95% R.H.	34% R.H.	95% R.H.
	15	44	6	26	24	45	5
	30	45	5	22	28	44	6
		Disturbed				Disturbed	
	45	43	7	18	32	41	9
1	0	41	9	14	36	38	12
1	15	37	13	13	37	35	15
1	30	—	—	14	36	—	—
1	45	—	—	10	40	—	—
				Disturbed		—	—
2	0	—	—	21	29	—	—
2	15	—	—	16	34	—	—
2	30	—	—	14	36	—	—

The taxis

This reaction is well shown by ticks climbing a glass rod. An example is given in Fig. 9. In a uniform humidity of 95% R.H., this unfed female tick showed a slight tendency to turn upwards after walking down the vertical rod for a variable distance (Fig. 9 A). With the upper 8 cm. of the rod exposed to a humidity of 34% R.H. and the lower 16 cm. to 95% R.H., the tick executed the most regular series of avoiding reactions, almost invariably turning upwards from the moist air near the point where the humidity gradient is steepest. The track is therefore confined to the dry air at the tip of the rod. Under these conditions the tendency to turn upwards on the vertical rod and the avoiding reaction reinforce one another. If the apparatus is inverted so that the upper part of the rod is exposed to 95% R.H., the response is much less regular (Fig. 9 C). The tick may in fact turn upwards at any point on the rod, and although a few avoidances at the humidity boundary result in downward

turning movements, very often the tick progresses upwards rather hesitantly into the moist air.

The typical response at the humidity boundary is very simple. The tick stops abruptly or even shrinks back, waves its forelegs more violently than usual, then turns round and walks away. The response is sometimes less perfect. In Fig. 9 B there is one faulty orientation where the tick stopped, moved round the rod but continued walking downwards. Some individuals previously exposed to saturated air may fail to show good avoiding responses, and the taxis invariably fails also if the ticks are walking with their forelegs or are running in an agitated manner.

The previous experiments had indicated that the state of water balance greatly influences the expression of the avoiding reaction. This was confirmed by selecting individuals showing a strong humidity response and desiccating them progressively.

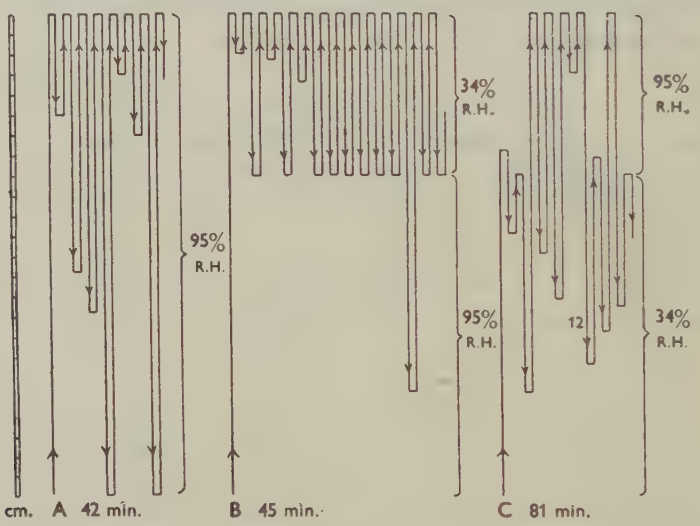

Fig. 9. Tracks of the same tick on a vertical rod showing the reaction to alternative humidities. A, movements in a uniform humidity of 95 % R.H.; B, C, tracks with alternative humidities of 95 and 34 % R.H.

A horizontal rod was used in order to avoid the turning effects which occur when the rod is vertical. Fig. 10 shows an unfed female tick which responded very clearly to alternative humidities of 34 and 95 % R.H. After desiccation for only 2 hr. in dry air at 25° C. the response to these alternative humidities had almost vanished and there remained only an occasional avoidance of 95 % R.H. or a slight hesitancy on crossing the boundary (Fig. 10 B). Yet after exposure to saturated air for 24 hr. the response is restored to its original strength (Fig. 10 C). This example illustrates the sensitivity of the taxis to the changed physiological state, for during the period of desiccation the tick is unlikely to have lost more than 1 % of the original body weight (Lees, 1946). Two other unfed female ticks, when offered humidities of 34 and 100 % R.H., continued to show good avoiding reactions after 2 hr. in dry air at 25° C. (four out of four tests on the rod were positive), but after 8 hr. these became hardly perceptible (seven out of eight tests were negative).

The expression of the taxis with other humidity differences was also investigated. About six ticks, selected for the humidity response, were tested individually on a horizontal rod. Care was taken to guard against any possible effects of desiccation by returning them to saturated air for at least 24 hr. after each trial with different humidity alternatives. The results are summarized below and in Fig. 11.

The higher alternative humidity was avoided consistently in the following combinations: 95/86%; 95/78%; 95/67%; 95/44%; 95/34%; 92/78%; 92/67%; 86/67%; 86/44%; 86/34%; 78/34% R.H.

The higher alternative was sometimes avoided with humidities of 95/92%; 92/86%; 78/67%; 78/44% R.H.

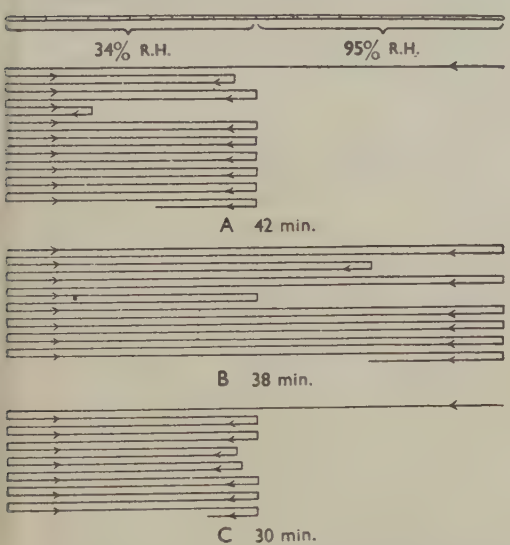

Fig. 10. Movements of the same unfed female on a horizontal rod when exposed to high and low alternative humidities. A, normal water balance; B, after desiccation for 2 hr. in dry air at 25° C.; C, after subsequent exposure to damp air for 24 hr.

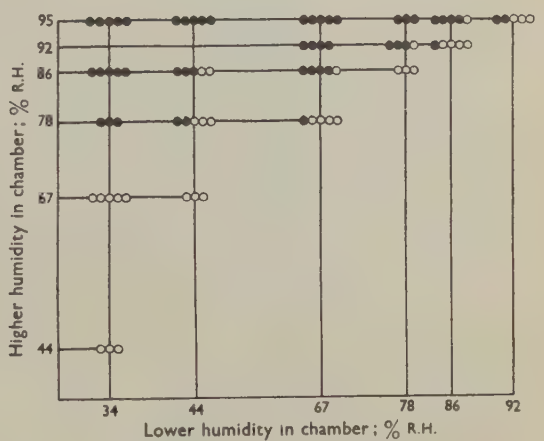

Fig. 11. Chart illustrating the avoiding reactions (taxes) of unfed ticks when offered different pairs of alternative humidities on a horizontal rod. A successful avoidance of the higher humidity is indicated by a black circle; the absence of such a response by an open circle.

No avoidance of the higher alternatives was shown with humidities of 86/78%; 67/44%; 67/34% and 44/34% R.H.

There were no undoubted examples of avoidance of a lower humidity.

These results show that for a given humidity difference the response is most intense at high humidities. Thus, whereas there was a clear response with humidities of 95 and 92% R.H., lower down the humidity scale no response was elicited with a humidity difference as large as 33% R.H. (e.g. with alternatives of 67 and 34% R.H.). It may be recalled that several insects, including the mosquito, *Culex fatigans* (Thompson, 1938), the adult mealworm *Tenebrio* (Pielou & Gunn, 1940), the louse *Pediculus* (Wigglesworth, 1941) and the wireworm *Agriotes* (Lees, 1943), also discriminate most accurately between small humidity differences when the air is relatively moist.

Desiccated ticks were also exposed individually to various alternative humidities with differences ranging up to 66% R.H. As had previously been suspected, they fail to avoid either higher or lower humidities. In a typical example (Fig. 10 B) the tick crosses the gradient in either direction without hesitation.

The kinesis

The preliminary experiments have suggested that the movements of desiccated ticks are soon arrested in moist air and that this mechanism plays a most significant role in the humidity behaviour. Further light has been thrown on the kinetic response by observing the behaviour of sets of twenty-five unfed nymphal ticks in small activity chambers. The latter were similar to the alternative chambers, but

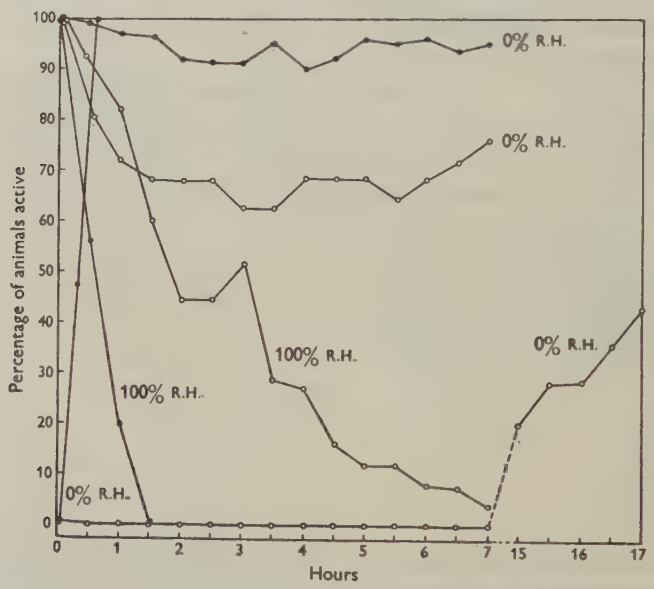

Fig. 12. The kinetic response to humidity. The figure indicates the progressive rise or fall in activity among sets of unfed nymphs exposed to dry or moist air. o——o, normal ticks from damp air; •——•, desiccated ticks.

were undivided; only uniform humidities of 0 or 100% R.H. were employed. The ticks used had either been exposed previously to saturated air for some days or had been desiccated overnight at 25° C., 50% R.H. The number of active ticks were recorded at 15 min. intervals, starting from the moment of introduction into the chamber. At the beginning of each experiment therefore every tick was active. The levels of activity following exposure to dry or moist air are shown in Fig. 12.

After mechanical disturbances the movements of normal nymphs in saturated air were arrested gradually, 92% having settled down after 6 hr. In dry air akinesis begins to develop as rapidly as in saturated air, but after about 1 hr., as desiccation begins to take effect, the number of ticks at rest increases no further and soon begins to decrease. After desiccation, nymphs exposed to dry air rarely come to rest and usually continue to walk round the chamber until they die from excessive water loss.

The activity of the desiccated ticks in moist air is in complete contrast: locomotion ceased so rapidly that after 1 hr. 15 min. every tick was quiescent and most had folded their legs. Only 40% of the normal, undesiccated ticks came to rest in saturated air within a similar period.

Also included in Fig. 12 are the results of the converse experiment where normal or desiccated ticks were exposed to dry air whilst at rest. This was accomplished in the following way. Normal ticks were allowed to settle down in the moist chamber overnight. After such an interval nearly every individual comes to rest and in humid conditions remains so almost indefinitely. The voile arena with the upper half of the chamber could then be lifted gently on to another Petri dish containing concentrated sulphuric acid. Desiccated ticks were exposed to saturated air in the chamber for a period (usually about 1 hr.) sufficient to ensure complete inactivity, but of insufficient length to permit any significant replenishment of the depleted water balance. Dry air was then substituted for moist. The effects of desiccation are most striking, for these nymphs are almost immediately aroused to violent activity in dry air: indeed, after 30 min. every tick was walking round the chamber. Normal ticks remained inactive for at least 6 hr. and even after 15 hr. only 20% had become active. After this the level of activity increased progressively.

These results may be summarized by stating that normal ticks tend to come to rest in dry air as readily as in moist air; that desiccated ticks come to rest very rapidly in moist air, but are stimulated to continuous activity in dry air; and that ticks already at rest are only aroused in dry air when desiccation becomes acute.

It would be difficult to decide the extent to which the water balance must be depleted in order to hasten arrest in saturated air. It is much more simple to follow the effects of water loss on the subsequent activity of ticks already at rest. For this purpose, five unfed females were allowed to settle overnight in saturated air. Each was now exposed to dry air and was removed and weighed on a torsion balance as locomotion began. At room temperature of about 15° C., the average period of inactivity was 49 hr. and the percentage loss of weight 12% (maximum 15%, minimum 9%). The depletion of the water balance necessary to provoke activity is thus much more severe than that required to eliminate the avoiding reaction.

Evidence contained in the section on the sense organs (p. 189) indicates that the kinetic response persists after the forelegs, which bear the main sensory equipment, have been amputated. It therefore seems unlikely that the kinesis is associated with any particular type of receptor. In woodlice, which show a well-defined kinetic response (Gunn, 1937; Waloff, 1941), no hygroreceptors are known, but the behaviour differs in that the kinesis comes into operation immediately after exposure to high or low humidities. In *Ixodes* it is the perception of continuing water loss when the water balance is already depleted and of cessation of such loss, that leads respectively to activity or akinesis.

The mechanism of the kinesis is uncertain. There may be some direct association with the progressive concentration or dilution of the haemolymph, but it may well be that the mechanism is more subtle. We have previously referred to the fact (Lees, 1946) that unfed ticks which have lost water by evaporation can take up

water through the cuticle from damp air. While the tendency of the unfed tick to come to rest in damp air after desiccation may at first be connected with the arrest of evaporation, a later influence appears to be the perception that the epidermal cells are successfully engaged in taking up water. This is suggested by the following observations on the rate of development of a kinesis in saturated air after successive acts of disturbance. Batches of twenty-five unfed nymphs were desiccated, exposed to saturated air and allowed to come to rest. The quiescent ticks were then aroused to activity on successive occasions by dislodging them from the walls and floor of the chamber. As Fig. 13 shows, the movements are arrested more and more rapidly after each act of disturbance. This is very probably associated with the progressive development of secretory activity and of water uptake through the cuticle. It cannot

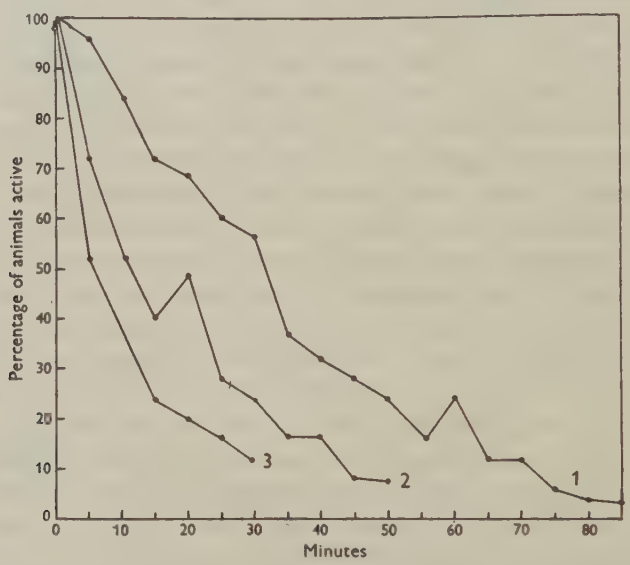

Fig. 13. The progressive development of the kinesis in a single batch of desiccated ticks in saturated air. After coming to rest the ticks were aroused by mechanical stimulation. Note that the decline in activity becomes more rapid after each successive act of disturbance.

be due merely to the sensory adaptation resulting from successive tactile stimulations, as normal nymphs disturbed in a similar manner do not show the effect.

The foregoing account has emphasized the dependence of the humidity response on the physiological state, particularly the condition of the water balance. We may summarize the general behaviour as follows: when the water content is normal, the tick avoids high humidities but comes to rest with equal readiness in dry or moist air. After desiccation the taxis is replaced by a kinesis: the avoiding response disappears and movements are readily arrested in moist air and are provoked in dry air.

Hygrokinesis has been noted in several species of ticks. Totze (1933) offered *Ixodes ricinus* the choice of tubes containing dry or wet cotton-wool. No particular responses were seen with normal ticks, but after desiccation they collected in the

moist tube, settling on the wool. Young *Boophilus* larvae were indifferent to the choice of wet or dry tubes, but older larvae aggregated near wet cotton-wool although avoiding contact with it (Krijgsman, 1937). In a linear humidity gradient, unfed nymphs and adults, and replete females of *Dermacentor albopictus*, eventually come to rest at a humidity higher than 70% R.H. and the majority settle at about 85% R.H. (Howell, 1940). Clear evidence that the depletion of the water balance may cause a reversal of the direction of the humidity response was first given by Gunn & Cosway (1938) for *Blatta orientalis*. Some cockroaches spend more time in the dry region of a humidity gradient at a uniform temperature. On desiccation, the same animals become hygro-positive. The humidity behaviour of the sheep tick also closely resembles that of the beetle *Ptinus tectus* (Bentley, 1944). In a linear gradient these insects at first collect in the drier region, later dispersing and aggregating in the moist air. Bentley shows that this is the result of desiccation. Beetles provided with drinking water preferred dry air in an alternative chamber. Desiccated beetles, on the other hand, showed a weakened preference for dry air and a greatly enhanced tendency to become active in dry air and to come to rest in moist air. The reaction is again reversed when the beetles are allowed to drink. Behaviour of this type differs entirely from that of the louse *Pediculus* (Wigglesworth, 1941). In this insect changes in the direction of the reaction are associated with sensory adaptation, the louse at first tending to avoid humidities which it has not recently experienced. Adaptation cannot, of course, play any part in the reversed response of *Ixodes*, for after exposure to dry air (desiccation) the ticks aggregate in moist air; and after exposure to moist air (restoration of the water balance) the higher humidities are avoided.

The humidity behaviour of engorged ticks

The behaviour of sets of twenty-five engorged nymphs was followed in the small alternative chambers. These nymphs had all recently fallen from the host and were thus in a reasonably active condition. One batch of nymphs was used immediately after removal from saturated air; a second was desiccated overnight at 50% R.H., 25° C.; while a third was desiccated and subsequently exposed to saturated air at 25° C. for 48 hr. Results with alternative humidities of 34 and 95% R.H. are given in Table 4. Each experiment was replicated so that the number of position records at any moment of observation is fifty.

The reactions differ in some respects from those of unfed ticks (cf. Table 3). Normal engorged nymphs were present in almost equal numbers on the wet and dry sides of the chamber throughout the duration of the experiment. But there was a slight and significant excess on the dry side, as a few individuals showed avoiding responses when crossing from dry into moist air. It was noticeable that where the taxis failed the ticks were walking with the aid of the forelegs, as is usual after engorging, while those which responded were waving their forelegs.

The effects of desiccation develop more slowly than in the unfed tick which is much smaller, and consequently more susceptible. Nevertheless after desiccation overnight the behaviour is similar. At first, while they are circulating freely in the chamber, distribution is random; later, as the kinesis develops, the engorged ticks

begin to aggregate on the moist side (Table 4). Desiccated ticks subsequently exposed to moist air for 48 hr. continue to exhibit the kinesis, the tendency to settle down in moist air persisting. The absence of reversal of the humidity response after exposure to a saturated atmosphere is undoubtedly connected with the fact that the engorged tick loses the faculty, which the unfed tick possesses, of taking up water from damp air (Lees, 1946). Thus, after desiccation the engorged tick must remain with a depleted water balance and the kinesis remains also.

Table 4. *Distribution of fifty engorged nymphs in an alternative chamber providing low and high humidities*

A, normal ticks from saturated air; B, desiccated ticks; C, desiccated ticks subsequently exposed to saturated air for 48 hr. at 25° C.

Duration from start of experiment		A		B		C	
Hr.	Min.	34% R.H.	95% R.H.	34% R.H.	95% R.H.	34% R.H.	95% R.H.
	15	29	21	24	26	24	26
	30	28	22	20	30	21	29
	45	20	30	19	31	21	29
1	0	28	22	19	31	19	31
1	15	24	26	18	32	17	33
1	30	30	20	16	34	16	34
1	45	30	20	21	29	17	33
2	0	26	24	19	31	13	37
2	15	27	23	18	32	12	38
2	30	28	22	16	34	10	40
5	0	—	—	8	42	—	—

REACTION TO TACTILE STIMULI

The ability of ixodid ticks to cling to moving objects is shown very strikingly by *Ixodes* nymphs which have climbed and settled near the tips of glass rods. If a piece of cotton-wool is brought up to the rod, the questing tick catches hold and transfers itself to the wool with wonderful agility. Nymphs with their legs folded are aroused by contact with the wool and are carried away in a similar manner. On a neutral object, such as the cotton-wool, the tick soon loses interest and drops off. The behaviour on the warm, scented skin of a mammalian host is quite different: before arriving at an attachment site the tick clings on tenaciously.

The response to vibration and movements of the substratum is well displayed also by nymphs which have come to rest near the tips of glass rods with their legs folded. If the rod is tapped gently the tick responds instantly by questing. It may continue to remain in a rigid questing attitude for some time before folding the legs again, or, if greatly disturbed, may begin walking round the tip questing. Ticks become adapted to a fairly regular mechanical disturbance. For example, when the bench on which the rod stands is tapped regularly with the forefinger questing gradually becomes less vigorous and the attitude of repose is resumed. If now, while the tapping is continued, the rod itself is touched, the tick quests again instantly. The stimulating vibrations evidently have a different quality. This circumstance is of some significance in the natural environment (p. 202).

Ticks also respond to moving air. If a jet of air is played on an unfed tick while it is climbing a rod, it attempts to shelter from the wind by walking round to the leeward side. On a plane horizontal surface unfed ticks are deflected from their course by a jet of air not sufficiently strong to dislodge them. Positive anemotaxis to a slight puff of air (Totze, 1933) was not observed.

The response to contact with the bodies of other ticks is very noticeable in the unfed larva. If a number of larvae are distributed in a tube, the majority finally come to rest in a dense clump, many layers deep. The ticks are oriented in different directions in such a manner as to fit into one another with the greatest economy of space. If the tube is tapped or warmed with the hand, the cluster partly disperses, the ticks near the periphery wandering off separately. Usually a group of larvae remain near the centre and the clump gradually reforms as the isolated larvae encounter the cluster again and come to rest. The aggregating behaviour of tick larvae has been noted by many authors (e.g. Krijgsman, 1937). The response is much less evident in unfed nymphs and still less so in the case of adults.

The reactions of the sheep tick to contact with other objects are not pronounced, but Totze (1933) noted that when unfed ticks were offered several different materials on which they might come to rest, the order of preference was smooth paper, filter paper, flannel and lastly cotton-wool. Engorged ticks also develop a strong inclination to insinuate their bodies into crevices.

REACTION TO TEMPERATURE

Token response to a warm tube

Several blood-sucking insects, of which *Rhodnius* (Wigglesworth & Gillett, 1934*a*) and *Cimex* (Sioli, 1937) are examples, are attracted by objects at blood temperature. This is also true of ticks: Krijgsman (1937) pointed out that *Boophilus* larvae oriented to the warm (34° C.) arm of a T-shaped tube; and Totze (1933) mentions that hungry *Ixodes ricinus* will follow a warmed tube. The response in *Ixodes* has been reinvestigated by observing the behaviour of ticks advancing towards a test-tube through which warm water (usually 37° C.) was circulated. In the usual arrangement the tube rested vertically on a large sheet of plate glass. At first the ticks were set down on the glass about 4 cm. from the tube and were allowed to wake up spontaneously. If they walked away from the tube or passed it at such a distance that the stimulus was not perceived, the experiment was repeated. Later it sufficed merely to set the tube in the path of the tick. The best orientations are seen when the tube is approached obliquely. Since the stimulus is perceived only when the tick is very close to the source, the use of two tubes, representing alternative stimuli (Wigglesworth & Gillett, 1934*a*), is impracticable.

Some specimen temperature gradients around the tube were plotted with the aid of a sensitive resistance thermometer (Skin Temperature Recorder). With the tube containing water at 37° C. and room temperature at 25·5° C., the following temperatures were recorded with the sensitive junction of the instrument resting against the floor:

Distance from tube (cm.)	0·0 (touching)	0·2	0·5	1·0	2·0	5·0
Floor (° C.)	34·6	28·8	26·4	26·2	25·8	26·0

2 mm. above the floor (approximately the height at which the forelegs are carried) air temperatures did not differ from the above by more than 0·4° C.

Results with fifty hungry females, nymphs and larvae are summarized in Table 5. The stimulus provided by the warm odourless tube can be classed as 'attractive' or 'repellent' according to the final response in climbing on to the tube. Unfed females responded to the tube only on approaching to within 1 cm. or less of the base, absence of perception at greater distances being shown by the undeviating course pursued and by the regular movements of the forelegs. Of these fifty ticks, thirty-nine avoided the tube. After approaching, the tick stops or shrinks back, waves the forelegs more violently than usual, then turns round and walks away (Fig. 14 A). Or it may merely be deflected from the original course and continue without stopping. Eleven females, on the other hand, were attracted to the tube. The complete response is characteristic. As soon as the stimulus is perceived, the tick waves its forelegs in the direction of the tube, orients, runs up to it, swiftly climbs on, and runs excitedly over the surface (Fig. 14 B). Other types of behaviour are intermediate between full repellency and attraction. Some ticks orient to the

Table 5. *Reactions of hungry ticks to a warm tube in the presence or absence of sheep wool*

Stage	No. of ticks	37° C. odourless		37° C. wool		20° C. wool	
		No. attracted	No. repelled	No. attracted	No. repelled	No. attracted	No. indifferent
Females	50	11	39	40	10	6	44
Nymphs	50	48	2	49	1	17	33
Larvae	50	48	2	48	2	6	44

tube, and, advancing towards it, stop at the base to investigate the tube with the forelegs, finally climbing on rather hesitantly. Others, after running up to the tube, stop short of the base and avoid it strongly (Fig. 14 C). It seems clear that the attractive and repellent qualities of the stimulus are very evenly balanced. When the same individual is tested on different days, the warm tube is recognized as a 'host' on some occasions but is avoided on others. Rough handling is sufficient to lead to consistent avoidance. Further, ticks which are attracted in their first excitement at perceiving the warm tube may later come to exercise greater discrimination. The tracks of such a female at successive trials are shown in Fig. 15. At the first two approaches to the tube the tick climbed on eagerly and was removed carefully on a slip of paper; afterwards it began to avoid the tube strongly.

The behaviour of the male is identical with that of the female tick; for the immature stages, however, temperature is of relatively greater significance in orientation to the host. Indeed, placing the warm tube among a cluster of larvae is not unlike introducing one pole of a magnet among a heap of iron filings. Forty-eight out of fifty hungry nymphs and the same number of larvae were attracted by the tube and only two were repelled (Table 5). The same is true of *Rhodnius*, for whilst temperature is of outstanding importance to the young nymph, the adult

makes use of the additional stimulus of smell in locating the host (Wigglesworth & Gillett, 1934*a*).

The token response to temperature is closely related to the nutritional condition. Unfed ticks which have only recently moulted (up to 2 months or so after emergence in the case of adults) are not hungry and consistently refuse to approach the tube. They are never attracted until their reserves are substantially depleted. Fully engorged ticks are always strongly repelled by a tube at 37° C.

The response was also investigated when the temperature of the warm 'finger' and that of the general environment are varied. The ticks were allowed to walk on the flat top of a copper tank through which water at the required temperature could

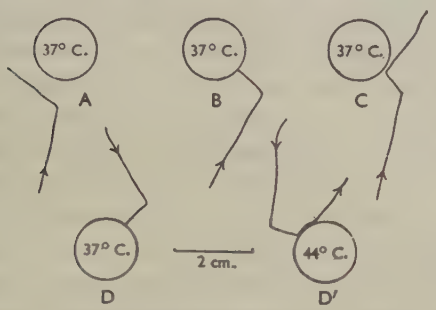

Fig. 14. Tracks of hungry ticks approaching the base of a warm odourless tube. A–C, females; D, D', the same nymph.

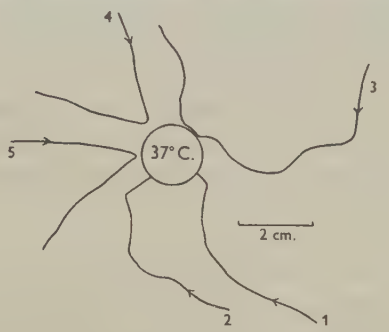

Fig. 15. Consecutive tracks of a single hungry female tick approaching the base of a warm odourless tube.

Table 6. *Reactions of three unfed nymphs to a warm tube when the temperature of the floor and that of the tube were varied independently*

o = indifferent; + = attracted; − = repelled; ± = attracted, then repelled.

Temperature of floor (° C.)	Temperature of tube (° C.)						
	14	18	20–21	25	30	36–37	43–44
18	o o o	o o o	+ + −	+ + +	+ + +	+ + +	± ± −
24	o o	o o	o o	+ o o	+ + +	+ +	− −
31	.	.	o o	.	o o o	+ − o	± − −

be circulated. The 'finger', a 3 × 1 in. flat-bottomed specimen tube, containing a thermometer, was circulated independently. This tube was clamped in a retort stand in such a way as to slide easily over the surface of the tank. Unfed nymphs were used, as they are consistently attracted by warmth; and because of their small size and depressed bodies it is reasonable to assume that the body temperature is the same as that of the surface of the tank. The usual procedure was to slide the tube into a position where the tick would pass it obliquely at a distance of about $\frac{1}{4}$ cm. Or, under certain conditions where the stimulus was expected to be repellent, it might be set directly in the path of the tick. Three types of response were recognized: the tick may be indifferent, failing to orient to the tube; it may be attracted and climb on to the tube; or it may be repelled. Records obtained with three individuals are set out in Table 6.

When the temperature of the finger is the same or lower than that of the floor, the tick is indifferent and walks round the base without climbing on. If the finger is higher than the floor temperature, the nymphs are often attracted from the very close distances. Indeed, one mild but undoubted response occurred with finger and floor temperatures of 25 and 24° C. respectively. The absolute temperature of the finger, provided it is below 40° C., is of no significance in determining the type of response, although naturally the vigour of the orienting movements and the distance at which the stimulus is detected are less when the temperature differences are smaller. With a finger temperature of 43° C., on the other hand, all the ticks were repelled: some appeared to avoid the tube as soon as the stimulus was perceived; others oriented to the finger and ran towards it, but departed hurriedly after halting abruptly at the base (Fig. 14 D, D′). In such instances it is clear that, although the ticks are responding to a temperature gradient around the finger, the absolute temperature at some point within the gradient may determine the type of response.

In general, ticks climbing on vertical glass rods react in a very similar manner to a gradient of warm air. Four unfed nymphs which had climbed to the tips of the rods and were already questing were tested by bringing up the rounded end of a tube containing water at 37° C. to within a distance of 0·5 cm. All the nymphs oriented strongly and attempted to reach and climb on to the tube, 'rearing up' and waving the first and second pair of legs. Often their excitement was so intense that they lost their foothold and fell from the rod. Four nymphs which had settled near the tip with their legs folded, quested immediately and began to move about when the warm tube was brought up.

Reactions in a linear temperature gradient

In investigating the reactions of *Ixodes ricinus* to temperature Totze (1933) employed a circular gradient around a warmed element in the centre of the arena. MacLeod (1935) made use of a linear arrangement consisting of a copper strip cooled at one end and warmed at the other. In the present work the linear gradient apparatus described by Wigglesworth (1941) was used. This consists essentially of an inner zinc trough 45 cm. in length and 4 cm. in depth, embedded deeply in a larger outer trough containing sand. One end is cooled by laying blocks of ice on the sand while the other is warmed from beneath by means of a series of graded Bunsen flames. In order to adapt the apparatus to its present use, the ticks were confined in a glass tube 42 cm. long and 0·75 cm. in diameter which could be laid inside the inner trough. Floor temperatures were recorded by twelve thermometers inserted in the sand and evenly spaced along the sides of the trough. The gradient inside the tube was checked against the recorded floor temperatures by passing a thermometer inside. With this apparatus it was possible to maintain a fairly even and steady gradient of about 0·9° C./cm. extending from 8 to 45° C. Fifty unfed or twenty-five engorged nymphs were introduced into the tube, then saturated air was drawn through and the ends corked. The numbers of ticks collecting in the several sections were noted after 15 min. following which the ticks were redistributed. Each experiment was repeated eight times.

Of the batches of unfed nymphs tested in the gradient, one had previously been kept at 25° C. for 2 weeks while the other consisted of wild nymphs taken in April by dragging a blanket over an infested grazing. The latter, which had thus been exposed to outside winter temperatures, were kept out of doors until required for experiment. During the two weeks prior to collection the average of the daily maximum and minimum temperatures recorded by a thermograph housed in a standard screen were 13·5 and 5° C. respectively. The chill-coma temperature of the ticks exposed to 25° C., as determined by the method of Mellanby (1939), was 7° C.; and that of the wild ticks about 1° C.

The results showed that unfed ticks previously exposed to warm conditions aggregate very rapidly in the extreme cold end of the gradient, 75 % being recovered from the 8–11° C. section (Fig. 16 A). Very few ticks are present above 30° C. This

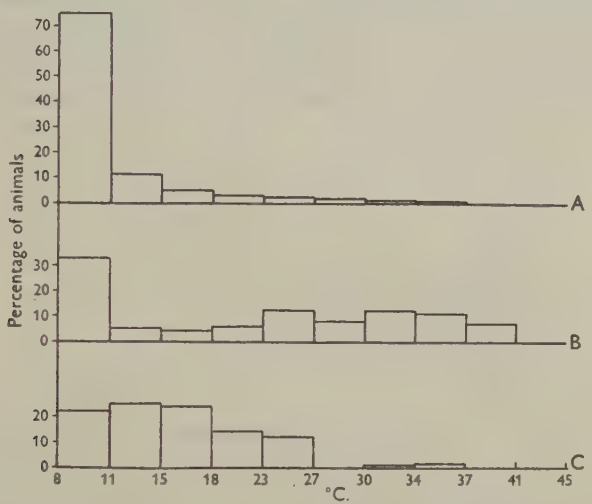

Fig. 16. Average distribution of ticks in a temperature gradient. A, unfed nymphs previously exposed to 25° C.; B, wild unfed nymphs from an infested grazing (cold-adapted ticks); C, engorged nymphs previously exposed to 25° C.

distribution is partly the result of the reactions displayed in the warmer parts of the gradient. A tick advancing up the gradient gradually accelerates its pace before turning abruptly and walking in the opposite direction. But it is also partly due to the lack of any such response as the cooler end of the tube is approached. As it moves into cooler air the rate of locomotion merely slackens until, at the coldest point, the nymph becomes almost completely immobilized. Had the experiment been continued for a longer period all the ticks would have been trapped eventually at the cold end. The same asymmetrical distribution obtains with the nymphs taken from the blanket although, since they are adapted to colder conditions and can progress slowly even at the lowest temperatures, many fewer become trapped (Fig. 16 B). Among the ticks remaining outside the coldest section there are no well-defined aggregations at any temperature from 11 to 41° C. and there is thus no indication of any definite 'preferred temperature'. Engorged nymphs previously

exposed to 25° C. show the same trends in distribution but, perhaps because they move more sluggishly, only 22% had collected in the coldest section before the experiment was terminated (Fig. 16 C).

These results do not confirm the findings of Totze (1933) or MacLeod (1935), both of whom regard the sheep tick as having a well-defined thermal preferendum. Although a significant number of ticks were trapped in the cold, MacLeod recorded the largest numbers at 14–17° C. Totze conditioned unfed ticks of all stages at temperatures ranging from 11 to 19° C. and claimed that the preferred temperature, which is shown in many of his figures as a sharply defined zone, varies from 15 to 19° C. after this treatment. Investigations on the 'preferred temperature' of many insects have shown that distribution within a linear gradient is uni- or bimodal with the largest aggregations commonly occurring in the coldest regions as the result of trapping (Deal, 1941; Gunn & Walshe, 1942). In *Ixodes* this effect is largely artificial, being brought about by high adaptation temperatures, although it may be assisted by the linear form of the apparatus. The significance of such results in terms of the behaviour under natural conditions is open to question (see p. 201).

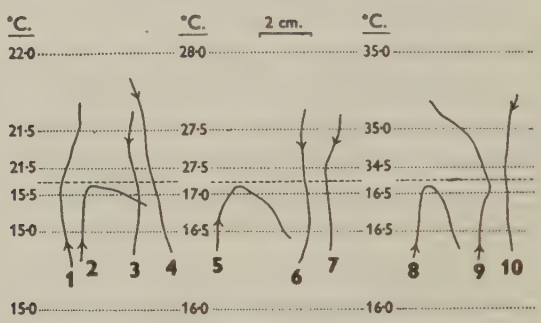

Fig. 17. The reactions of ten hungry female ticks to 'alternative temperatures'. Only parts of the track where the ticks are crossing the steepest region of the gradient (interrupted line) are shown. Isothermals are indicated by dotted lines. Examples are given of indifference to a higher temperature (no. 1), of indifference to lower temperatures (nos. 3, 4, 6, 7, 10), of avoiding responses to higher temperatures (nos. 2, 5) and of attraction to a higher temperature (no. 9).

Reactions to 'alternative temperatures'

We have already mentioned that no avoiding reactions to lower temperatures were seen in the linear gradient, although there were many such reactions to higher temperatures. In order to confirm this conclusion the behaviour of single ticks crossing a relatively steep temperature gradient was studied. The apparatus used, which was similar to that described by Wigglesworth (1941), consisted of two copper tanks 20 × 20 × 9 cm. held and bolted together by means of an outer metal strip. An asbestos sheet between the tanks provided the insulation. Each tank could be circulated independently with water: usually one was cooled by pouring in iced water while the other was warmed with a Bunsen. The arena of voile stretched on a wire ring rested half on one tank and half on the other. There was no wall enclosing the arena, and the ticks, previously exposed to 25° C., were set down facing the temperature boundary, the tracks being followed only within the im-

mediate neighbourhood of this zone. The gradient was plotted with the Skin Temperature Recorder and is indicated in Fig. 17. Room temperature was about 21° C.

Some of the tracks obtained with alternative temperatures are given in Fig. 17. Most female ticks crossed from 15 to 21° C. without hesitation, but a few avoided the boundary and returned to the cooler side. Many ticks crossing from 15 to 27 or 34° C. turned and walked away before encountering the steepest part of the gradient. However, a few individuals were attracted, particularly if approaching the boundary obliquely, and, after orienting, hurried across to the warm side. The reactions are therefore very similar to those elicited by the warm tube. When the ticks were started from the warm side, on the other hand, they always crossed unhesitatingly into the cooler air, the rate of progression gradually slackening. With alternatives of 35 and 16° C. the temperature gradient at its steepest part was about 18° C./cm. yet no avoiding reactions were noted (Fig. 17).

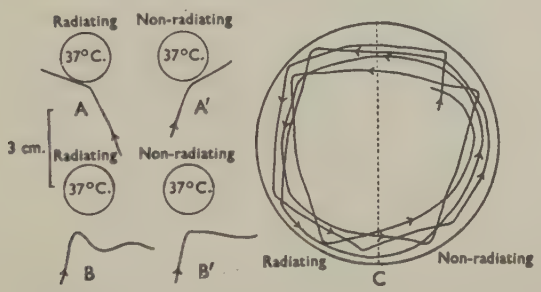

Fig. 18. The response to radiant heat. A, A′, B, B′, the same ticks (unfed females) approaching the bases of warm tubes with radiating and non-radiating surfaces; C, track of an unfed female in a divided chamber with radiating and non-radiating walls.

Response to radiant heat

In the past, the response of blood-sucking arthropods to objects at body temperature has frequently been assumed to be due to their perception of heat radiated from these objects; but where critical tests have been performed, as in *Rhodnius* (Wigglesworth & Gillett, 1934*a*, *b*) and *Pediculus* (Wigglesworth, 1941) air temperature and not radiant heat has been found to be the important stimulus. The very convenient method described in these papers of securing differences in surface radiation in the absence of differences in air temperature was employed in tests with *Ixodes*. Two similar test-tubes were covered with aluminium foil, one having a further covering of cellophane gummed on to the foil. Water at 37° C. was circulated through the tubes. As Wigglesworth (1941) points out, the emissivity of cellophane at this temperature is almost equal to that of a dull black surface while aluminium alone emits little more than 5% of this; yet the cellophane covering makes little difference to the gradient of air temperature around the tubes. Unfed female ticks, selected individually for their consistent avoidance of the warm tube, were observed approaching the two tubes consecutively. The examples shown in Fig. 18 A, A′,

B, B′, are typical: the tick approaches no nearer to the tube covered with aluminium foil than to the tube covered with cellophane.

Some observations were also made on female ticks walking in an arena with a cool floor and warm walls. The apparatus consisted of a tin so arranged that cold water circulated under the floor and warm water round the walls (Wigglesworth, 1941). The inner wall of the tin is lined half by aluminium and half by aluminium covered with cellophane. The temperature of the floor in the centre of the arena was 21° C. and that of the walls about 37° C. Ticks showing a consistent avoiding reaction as they approached the warm walls were repelled from the radiating and non-radiating surfaces at much the same distance (Fig. 18 C). The tracks obtained are reminiscent of those of the louse *Pediculus* (Wigglesworth, 1941).

We have already seen that hungry nymphs are consistently attracted by a warm tube. Unfed nymphs, which had come to rest near the tips of glass rods were tested for the response to radiant heat by bringing up successively the aluminium- and cellophane-covered tubes. After a response had been elicited, the tick was allowed to settle down again before the alternative stimulus was presented. The following are examples of the results. One nymph in repose quested vigorously when the non-radiating tube was $\frac{1}{2}$ cm. away; the same tick failed to respond when the radiating tube was farther away, but also quested when it was brought to within $\frac{1}{2}$ cm. Another tick with legs folded quested and oriented to the tubes only when the radiating and non-radiating surfaces were nearly touching its body. A tick which was motionless and questing responded by orientation when the non-radiating tube was $\frac{1}{2}$ cm. away and when the radiating tube was about $\frac{1}{4}$ cm. away. Finally, a nymph which was walking up the rod questing, oriented and tried to climb on to the tube when non-radiating and radiating surfaces were about $\frac{1}{4}$ cm. distant.

These results indicate clearly that ticks are repelled or attracted by air temperature and not by radiant heat. The same conclusion can be drawn from the fact that hungry nymphs become intensely excited when a slow stream of warm air, blown from a fine pipette 2 cm. distant, is allowed to impinge on them. They readily orient to the warm jet if it is directed laterally.

The response to temperature in the presence of sheep wool

The reactions of hungry ticks of all stages to sheep wool at 20° C. (room temperature) and to the simultaneous presence of wool and a warm tube at 37° C. are also recorded in Table 5. The freshly cut wool was wrapped closely round the base of the tube which contained either water at room temperature or circulating water at 37° C. As usual it rested on a glass plate over which the ticks walked. When both sheep wool and a warm tube were offered, forty out of fifty unfed females oriented and climbed on to the wool, often in a very excited fashion. The disturbance suffered in removal from the culture tubes probably accounts for the occasional failure of this stimulus to attract. Nymphs and larvae, which are much less easily upset, are almost invariably attracted when the warm tube and sheep wool are presented together (Table 5).

The attractiveness to females of warmth in the presence of wool contrasts with

the repellent qualities of warm air alone. That both temperature and smell are concerned in attraction is shown by the behaviour of single ticks when they are offered successively sheep wool at room temperature, a warm tube, and a warm tube plus wool. One example is shown in Fig. 19 A, A′, A″. The tick is indifferent to the wool at 21° C. and fails to climb on even if actually touching it. By itself the warm tube is strongly repellent but becomes attractive in the presence of wool.

The response to sheep wool at room temperature is not marked. Of the fifty females only six oriented to the wool and climbed on. Further, even this response was elicited only when the ticks were very close to the wool; and the behaviour is usually hesitating and lacking in excitement. Rather more nymphs were attracted to the wool from very close range, but few larvae showed any definite response.

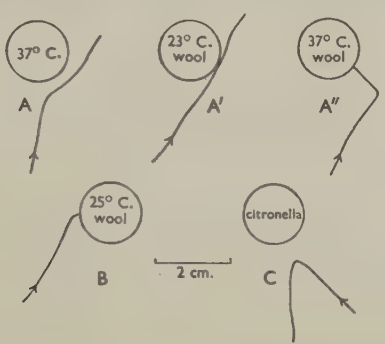

Fig. 19. The response to smell. A, A′, A″, tracks of the same hungry female approaching a warm odourless tube, a tube at room temperature with sheep wool wrapped round the base, and a warm tube in the presence of sheep wool; B, an example of attraction to wool in the absence of a temperature gradient (room and tube temperature 25° C.); C, unfed female avoiding the odour of citronella.

The same tests may be applied, with similar results, to unfed nymphs which had settled in repose near the tips of vertical glass rods. At 21° C. only one tick out of ten responded by questing when sheep wool was brought almost into contact with the forelegs. All the ticks quested and oriented when the warm tube alone, or a warm tube covered with wool was brought to within 0·25–1·0 cm.

RESPONSE TO SMELL

The previous statement that the hungry adult tick is indifferent to the odour of sheep wool in the absence of a temperature gradient, requires some qualification, for sheep wool proved much more attractive at higher temperatures even when there was no temperature difference between wool and the surrounding air. Twenty hungry females were tested with sheep wool wrapped round the test-tube in constant temperature rooms running at 6, 11, 19 and 25° C. They were first exposed to each temperature for one week to allow for thermal adaptation. At 6 and 11° C. none of the ticks oriented to the wool, even from very close quarters. At 19° C. two out of twenty were feebly attracted, while at 25° C. twelve out of twenty oriented quite strongly and climbed on to the wool (Fig. 19 B). This effect may be due to the

greater volatility of the wool odours at the higher temperature. Their significance in attraction will no doubt be even greater at normal fleece temperatures.

The reactions of ten unfed female and five male ticks to the odours of several mammals are analysed in Table 7. Freshly cut hair or fur was placed inside small caps of voile fitting over the rounded ends of a series of test-tubes. Since the response to smell is uncertain at low temperatures, it is necessary to provide a temperature gradient as well. The method of analysis has consisted in comparing the response of each individual to hair at 20° C. (no temperature gradient), to hair at 37° C. (temperature gradient present) and to a warm odourless tube at 37° C. (gradient present). All the tests were made at a room temperature of 20° C.

Table 7. *The reactions of hungry ticks to odours from the hair of several mammals in the presence or absence of a temperature gradient*

o = indifferent; + = attracted; − = repelled.

Sex	No. of ticks	Sheep		Dog		Horse		Cow		Rabbit		Odourless
		20° C.	37° C.	20° C.	37° C.	20° C.	37° C.	20° C.	37° C.	20° C.	37° C.	37° C.
Female	6	o	+	o	+	o	+	o	+	o	+	−
	1	+	+	+	+	+	+	+	+	+	+	−
	1	o	+	o	+	o	+	o	+	o	+	+
	2	o	−	o	−	o	−	o	−	o	−	−
Male	3	o	+	o	+	o	+	o	+	o	+	−
	1	+	+	+	+	+	+	+	+	+	+	+
	1	o	−	o	−	o	−	o	−	o	−	−

The results showed that the response to dog, horse or cow hair and to rabbit fur is much the same as to sheep wool (Table 7). The majority of the ticks are indifferent to the materials at 20° C., are repelled by the warm odourless tube, and are attracted by the material in the presence of a temperature gradient. One male and one female which were slightly attracted to sheep wool at 20° C., were attracted equally to the other materials in the absence of any temperature difference. The few individuals which were repelled by all the materials in the presence of a temperature difference, are without significance in the present context. The method of investigation does not permit any decision as to the relative attractiveness of the various materials, but no obvious differences were noted. Krijgsman (1937), on the other hand, is of the opinion that the larvae of *Boophilus* can distinguish different hosts by smell, the scent of cow or horse being preferred to that of man.

Little is known about the nature of the biological odours attractive to ticks, although Totze (1933) held that butyric acid was an essential constituent. In his experiments small pads of cotton-wool saturated with the test solution were placed in the centre of a large arena in which a number of ticks were liberated. A 1 in 100,000 solution was repellent, the ticks retiring to the edge of the arena. A 1 in 300,000 solution, however, provided an 'optimal concentration' of smell in the centre of the arena and most of the ticks then aggregated in this region. In view of the general interest of this statement these tests were repeated, but with entirely negative results. Small pieces of filter paper, moistened with saturated butyric acid

or with aqueous solutions prepared at dilutions of 1 in 2000 and 1 in 400,000 were inserted inside the gauze caps fitting on to test-tubes containing cold or warm water. At 20° C. concentrated butyric acid was powerfully repellent. The 1 in 2000 solution also proved mildly repellent. Ticks were indifferent to the weakest solution. In the presence of a temperature gradient all ticks tested were repelled as strongly from butyric acid at any of the three concentrations as from warm air alone.

The vapours of many substances, such as ammonia, naphthaline, formic acid, acetic acid, clove oil (Totze, 1933; Hindle & Merriman, 1912) act as powerful tick repellents under laboratory conditions. An example showing the repellency of citronella is shown in Fig. 19 C. Recent work of Smith & Gouck (1946) has demonstrated under field conditions the value of repellents such as indalone, dimethyl phtalate, etc. in reducing the attachment of *Ixodes scapularis*, and *Amblyomma americanum* to man.

REACTIONS TO LIGHT

Although it is well known that ticks, including eyeless species such as *Ixodes ricinus*, are sensitive to light, there is little agreement as to the nature or even the direction of the response. Hindle & Merriman (1912) showed that *Argas persicus* was always photonegative; with two light sources the ticks walked away along the resultant. Newly moulted larvae of *Boophilus annulatus* and *Rhipicephalus sanguineus* choose a darkened in preference to an illuminated tube, but older larvae become photopositive and go to the lighted half (Krijgsman, 1937). In very strong diffuse light *Boophilus* larvae remained photopositive while *Rhipicephalus* larvae retreated from the light. Totze (1933) examined the tracks of unfed *Ixodes ricinus* on a horizontal surface exposed to lateral illumination from a window, or from an artificial source. He stated that unfed ticks of all stages were strongly attracted to the light. In the case of dark-adapted ticks, however, the initial part of the track was somewhat convoluted, particularly with a very intense source of illumination. MacLeod (1935) repeated Totze's experiments, but was quite unable to confirm his results. All the ticks tested, whether unfed or engorged, were photonegative. Mironov (1939), working with the closely related species *I. persulcatus*, detected under conditions of low humidity a weak positive phototactic response at low intensities of illumination and a stronger negative phototaxis at higher intensities. In damp air the behaviour was always photonegative at all light intensities. These reactions were not influenced by previous exposure to light or darkness. All the above authors agree that engorged ixodid ticks invariably exhibit strong negative phototaxis.

Methods

The response to light has been investigated by following the behaviour of single ticks walking on a horizontal surface or climbing glass rods. The sources of illumination were shielded 60 W. bulbs or, in certain cases where higher intensities were required, a low voltage microscope lamp. In all cases the beam was first passed through a 3 in. tank containing a 2·5 % solution of iron alum acidified with sulphuric acid. Light intensities were measured with an Everett Edgcumbe photoelectric

cell. The intensities of the beam were adjusted according to need by inserting one or more sheets of thick paper into the lamp-holders. Light intensities below 10 m.c. (the lower limit of the instrument) were arrived at by extrapolation.

Response to changes in intensity of illumination

Although the location of the light receptive regions is not precisely known, one may conclude from the behaviour that light reaching a tick from the front or sides is directed, whilst illumination from the dorsal or ventral sides has only the attribute of intensity.

The response to changed light intensity is well shown by unfed nymphs which have come to rest near the tips of glass rods. A lamp directly above the rods gave a continuous surface illumination near the tips of 100 m.c. while an intense horizontal beam was arranged to give a light intensity of about 3000 m.c. at any point on the rod where the tick might settle. Care was taken to see that the switch controlling this lamp was not part of the bench fittings, for the vibration ensuing from pulling the switch was quite sufficient to cause the tick to quest. The following results are typical. If the tick is in repose with its legs folded, there is usually no response when the intensity is suddenly increased by 3000 m.c. But after exposure for some minutes to this light source, the tick quests instantly when the intense horizontal beam is switched off, and may start walking up and down the rod. Sometimes the response is less vigorous, with the tick perhaps slowly unfolding one leg before resuming the attitude of repose.

The vigour and sensitivity of the response to reduced light intensity seems to depend on the individual alertness of the tick, which, in turn, is influenced by temperature conditions. Fluctuating temperatures, such as prevail out of doors, favour an alert condition. The following example illustrates the sensitivity of the reaction in unfed nymphs kept outside in an open insectary. Seventeen active nymphs were present at the top of a tube which was being used to investigate activity. Light was reaching the tube from above and also from the sides. At 18.15 G.M.T. on 30 July 1946 the intensity of the vertical illumination was about 1000 m.c. When the intensity was reduced to 500 m.c. by shading the tube with a card (this was only just perceptible to the human eye), five of the ticks quested; when it was reduced to 100 m.c. by bringing the card closer, all seventeen ticks quested and began walking about.

Changes in light intensity have no influence on the behaviour of ticks which are not at rest.

Response to directed light

The method of investigation has usually consisted of exposing ticks which are walking on a horizontal sheet of paper with a surface illumination of 0·3 m.c. to a bright horizontal beam with an intensity of 500 m.c. Although the presentation of the directed beam is accompanied by an increase in light intensity, the effect of the latter can be ignored because, as we have already noted, this stimulus is without influence on the behaviour. The tracks were copied on a sheet of paper alongside.

Young female ticks (1 month after moulting) are strongly repelled by light and this behaviour is equally apparent whether the ticks are kept in darkness or are brightly illuminated for 1 hr. before the experiment. Older females (1 year after moulting) were much less sensitive to directed light and often continued to walk towards the horizontal beam when this was switched on (Fig. 20). Females walking at right angles to the horizontal beam pursued an undeviating course, however, and it is thus apparent that ticks approaching the light are not attracted but are merely indifferent to the stimulus. Further experiments with different horizontal light intensities gave similar results which are therefore in general agreement with MacLeod's (1935) findings, but are completely at variance with those of Totze (1933).

Engorged ticks invariably walk away from the light. Fig. 21 shows the tracks of a number of engorged ticks on a horizontal surface orienting to the horizontal

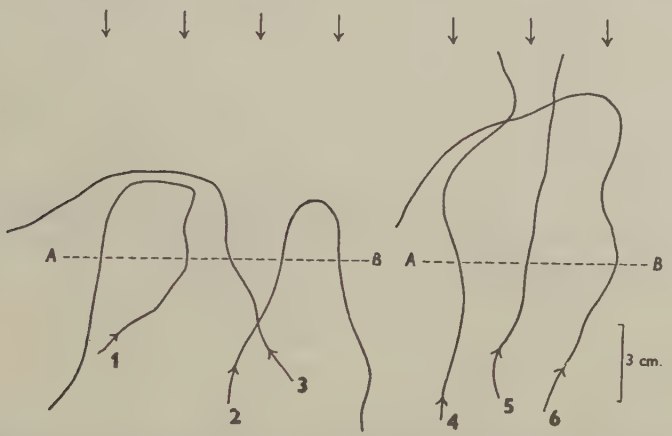

Fig. 20. Response to light. Tracks of unfed females walking on a flat horizontal surface dimly illuminated from above. On crossing the line *AB* the intense horizontal light is switched on. Nos. 1–3, females 1 month after moulting; nos. 4–6, unfed females 1 year after moulting.

beam. Orientation is sometimes most imperfect, for random turning movements are not always corrected until the track has deviated by as much as 160° from a path leading directly from the light source. More sensitive individuals reorient to the beam if the track deviates by as little as 10°. The importance in orientation of light reflected from the surroundings was shown by the behaviour of engorged nymphs in a circular arena 14 cm. in diameter and walls 1 cm. high lined by alternate segments of white and black paper. The arena was lighted from above so as to give a uniform surface illumination of 500 m.c. When the black strips were 5 cm. in length, the ticks nearly always avoided the white walls (Fig. 22): with strips only 3 cm. in length the deviations in the tracks towards the blackened segments were much less apparent. This response to reflected light ('skototaxis') has been described in many insects (Fraenkel & Gunn, 1940; Wigglesworth, 1941).

The intensity of the response to lateral illumination depends on the absolute intensity of stimulation in accordance with the Weber-Fechner law. This is shown

in Fig. 23 where engorged nymphs are exposed from above to light sources giving a uniform surface illumination of 0·3 or 500 m.c. As the ticks cross the line *AB* the horizontal orienting lamp, with an intensity of 10 m.c. at *AB* was switched on. It is clear that negative phototaxis is much more pronounced when the general intensity of illumination is low.

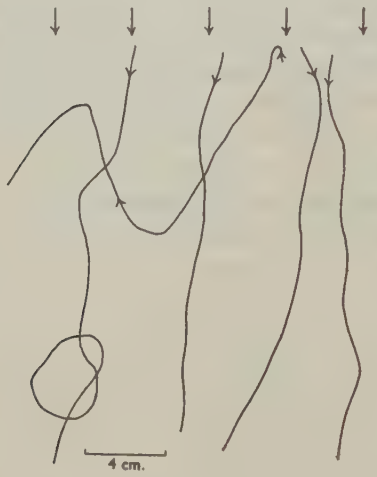

Fig. 21. Tracks of engorged nymphs orienting to a bright horizontal light.

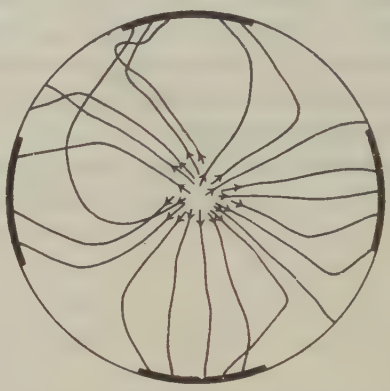

Fig. 22. Response to lateral illumination (skototaxis). Tracks of engorged nymphs in an arena with blackened segments on the walls. Vertical illumination of 500 m.c.

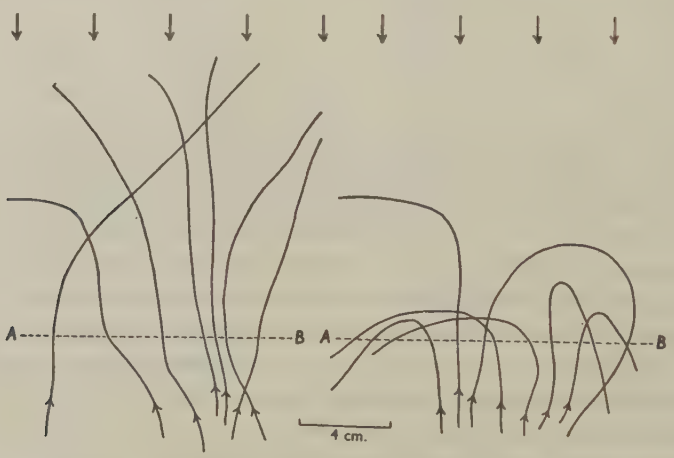

Fig. 23. Tracks of engorged nymphs orienting to a horizontal beam of 10 m.c. which was switched on as line *AB* was crossed. The group on the left were exposed to continuous vertical illumination of 500 m.c.; the group on the right to illumination of 0·3 m.c.

The nature of the response to light

It has always been assumed that in species of ticks which lack eyes, the response to light is mediated through a dermal light sense. And changes in the direction of the reaction have consequently been related to such events as the progressive

deposition of pigment in the cuticle after moulting (e.g. Krigjsman, 1937). Although some form of heat filter has been employed in most experimental work, the possibility remains that the ticks are reacting not to light but to the small amount of heat transmitted to the temperature receptors. This has been tested by comparing the response to a powerful source of radiant heat, the intensity of which can be varied independently of the light intensity, with the response to a source of light deficient in heating rays. The source of radiant energy, a small bowl fire with a good reflector, was connected with a suitable resistance so that the filament emitted no visible light. The light source was the horizontal beam of the microscope lamp which was filtered through the heat-absorbing tank. When their heating effects were compared by means of a small and sensitive thermopile, the amount of heating energy reaching the rod or arena from the light beam was found to represent about one twenty-ninth of that received from the fire at the normal working distances.

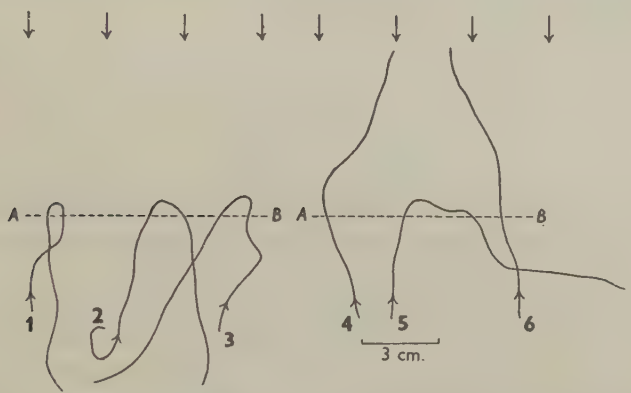

Fig. 24. Light and heat radiation as orienting stimuli. The figure shows the movements of six engorged nymphs under constant vertical illumination of 100 m.c. At *AB* nos. 1–3 were exposed to an intense horizontal beam of light; and nos. 4–6 to a powerful source of radiant heat.

(*a*) *The questing response.* These tests were made as usual on unfed nymphs which had come to rest at the tips of glass rods with their legs folded. A light vertically above the rods gave a constant surface illumination near the tips of 100 m.c. The observer was shielded from the ticks by a glass plate. The following results are based on observations on about ten ticks, but in several cases it was possible to follow the behaviour of the same individual throughout.

After a sudden increase in light intensity from 100 to 3000 m.c. the tick remains immobile with its legs still folded, whereas, after exposure to the intense horizontal beam for 10 min. there is an immediate questing response as this is switched off. If now, with the horizontal light off, the tick is exposed to the source of radiant heat by turning the bowl fire towards the rod, there is no *immediate* response, but after 15 sec. or so, the nymph slowly unfolds its legs and walks away. No doubt it is here responding to a gradual increase in body temperature. Some ticks eventually come to rest when exposed continuously to the fire. If the intensity of the radiant heat is suddenly reduced by turning the fire away, the tick remains with its forelegs folded

without responding. The questing reaction thus depends on the perception of reduced light intensities.

(*b*) *The orienting response.* Engorged nymphs walking on a horizontal sheet of paper under a constant vertical illumination of 100 m.c. were suddenly exposed to a horizontal beam of 3000 m.c. intensity or to the radiation from the bowl fire. The sheet of paper was rotated so that they approached each source head-on. Fig. 24 shows that the horizontal beam of light has a powerful orienting effect, the ticks soon retreating from it. The fire, although emitting an enormously greater amount of heating energy, provided only a feeble orienting stimulus, many ticks advancing towards it for considerable distances (Fig. 24, 4, 6). The few ticks which avoided it were no doubt showing the normal response of engorged ticks to warm air (p. 167). These observations also support the conclusions that there is a true dermal light sense. The mechanism of the response is presumably photochemical and is not due to the perception of minute heat differences.

THE SENSE ORGANS

The forelegs

(*a*) *Haller's organ.* First described in a species of *Ixodes*, this structure was regarded by its discoverer as an auditory organ equipped with otoliths (Haller, 1881). This interpretation was questioned by Lahille (1905), who suggested an olfactory function. Subsequently, other authors (Bonnet, 1907; Samson, 1909; Nuttall, Cooper & Robinson, 1908*a*) failed to find any trace of the 'otoliths' figured by Haller; and the later experimental work of Hindle & Merriman (1912) and Totze (1933) finally confirmed the impression that Haller's organ was concerned in the perception of odours. In their description of Haller's organ in *Haemaphysalis punctata*, Nuttall *et al.* (1908*a*) first drew attention to its composite nature: there are two main sensory regions, an anterior pit with short stiff bristles arising from the floor and a posterior vesicle or capsule enclosing a number of thin-walled and quite distinct sensory hairs. Recently, Schulze (1941) has examined the morphology of the cuticular parts of Haller's organ in a large number of species. Although there is some variability, the basic ground-plan is similar in all ticks. Schulze draws attention to the fact that bodies similar to those figured by Haller are sometimes present in the capsule. These 'Sekretballen' are described as yellowish balls of 'tallowy' material, insoluble in xylol; sometimes one or two are present, sometimes the capsule may be nearly filled with the finely divided material.

Haller's organ in *Ixodes ricinus* is shown in Fig. 25. The organ is situated on the dorsal side of the tarsus and is protected in front and behind by clumps of bristles—the anterior and posterior bristle groups (Fig. 25 A, C). Just behind the anterior bristle group lies an oval depression, the anterior pit (*g*). A number of sensilla, usually about seven in the case of the female tick, arise from the floor of the pit. The shape of these sensilla is distinctive; they are 8–25 μ in length, are very sharply tapered, slightly curved, and end in exceedingly delicate points (Fig. 25 C). Underlying the anterior pit is a compact group of sense cells from which a single nerve

arises. Each bristle in the pit is set in a small socket which in turn surmounts a canal penetrating the cuticle; fine processes, staining with haematoxylin, extend from the sense cells through the canals into the bases of the hairs.

The posterior capsule (Fig. 25 C, *h*) lies immediately behind the pit. The anterior and posterior walls are of thickened cuticle which is reflected upwards to form the delicate lateral walls and roof. The capsule itself is entirely enclosed save for a small

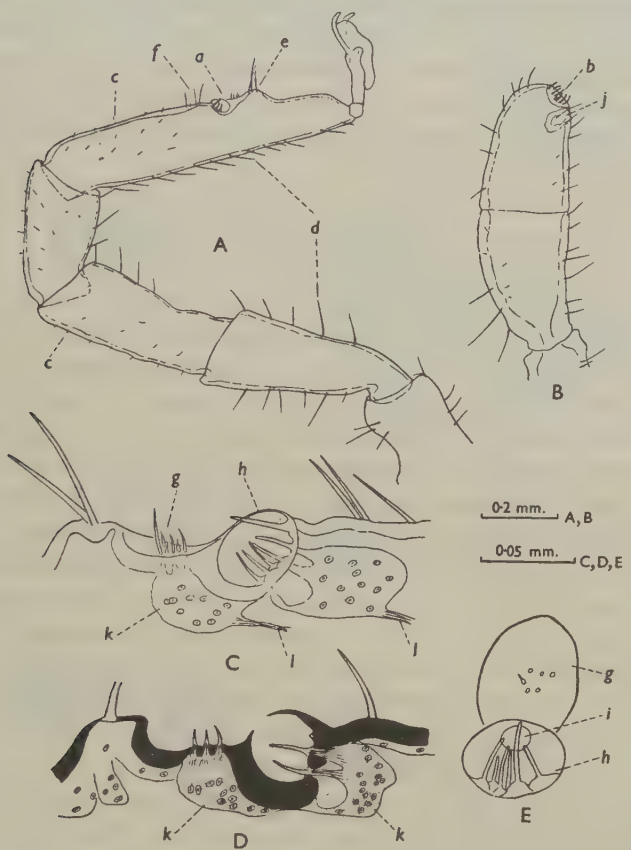

Fig. 25. A, foreleg of the female tick; B, ventral aspect of the palp; C, Haller's organ as seen in optical section of the leg; D, section of Haller's organ; E, central region of Haller's organ from the dorsal aspect; *a*, Haller's organ; *b*, palpal organ; *c*, temperature sensillum; *d*, tactile bristle; *e*, anterior bristle group; *f*, posterior bristle group; *g*, anterior pit; *h*, posterior capsule; *i*, aperture in roof; *j*, 4th palpal article; *k*, sense cells; *l*, sensory nerve.

circular opening in the roof which is difficult to detect except from the dorsal aspect (Fig. 25 E, *i*). In the female tick the capsule contains about six sensilla inserted on slight elevations along the floor and posterior wall. These are of the typical 'chemoreceptor' type, thin-walled, slightly curved and with blunt, rounded ends. Most of the bristles are completely enclosed within the cavity of the capsule, but one long straight sensillum arising from the dorsal region (the 'Signalhaar' of Schulze, 1941) projects through the aperture in the capsule roof to the exterior. Schulze

suggests that the opening in the roof can be occluded by certain movements of the leg, but this seems unlikely as the walls of the capsule appear completely rigid. In sections of the organ a further cluster of bipolar sense cells, quite distinct from that underlying the anterior pit, can be distinguished beneath the capsule (Fig. 25 D, *k*). Numerous terminal processes extend from these cells into the cavity at the base of each bristle. The innervation has not been followed in detail, but in some unstained whole preparations the groups of sense cells were seen to be provided with separate sensory nerves (Fig. 25 C, *l*). The independent innervation of the anterior pit and posterior capsule favours the view, which we shall consider later, that their sensory functions also differ. The anterior and posterior bristle groups are also innervated by groups of underlying sense cells. These bristles do not differ appreciably from the tactile bristles described below.

About twenty tarsi were mounted in canada balsam or polyvinyl alcohol for examination of Haller's organ, but in none of the capsules could the 'Sekretballen' of Schulze (1941) be distinguished with certainty. Some of the capsules were empty; others contained definite particles of dirt which had evidently entered through the aperture in the roof (no doubt the cleaning operations (p. 185) assist in keeping the capsule and the bristles in the pit free from contamination).

(*b*) *Tactile bristles.* These are thick-walled bristles over 100μ in length, often slightly curved and tapering to fine points. On the three distal articles of the leg they are confined entirely to the ventral surface, but on the proximal segments are present on the lateral and dorsal aspects as well (Fig. 25 A, *d*). The bristles on the tarsus are recumbent with their points directed towards the pulvillus. In the female tick about 100 tactile bristles are present on each foreleg. A section of this type of sensillum is shown in Fig. 26 C. The small socket-like structure in which the bristle is mounted rests at the end of a canal penetrating the cuticle. A fine sensory process (*b*), staining weakly in haematoxylin, can sometimes be detected within the canal. Although one or more nuclei lie at the base, these are indistinguishable from the nuclei of the other epidermal cells in the unfed tick. Neither trichogen nor tomorgen cells are visible. In *Dermacentor andersoni* the sensory cells and their nuclei may lie within the canal itself (Douglas, 1943), but in *Ixodes* the presence of sense cells can be detected with certainty only in regions where the cuticle is thickly beset with tactile bristles—beneath the anterior and posterior bristle groups, for example.

(*c*) *Small, straight bristles.* Although a certain number of bristles of intermediate size can be distinguished on the forelegs, the remaining hairs are, in general, of a uniformly small size in comparison with the tactile bristles. These sensilla are less than 50μ in length, and are usually both straight and very evenly tapered (Fig. 26 D). They are thick-walled, the small lumen appearing to extend only a short distance into the base. The distribution of these sensilla is the reverse of that of the tactile bristles for, although present on all the articles, they are almost entirely confined to the dorsal and lateral aspects of the leg (Fig. 25 A, *c*). In section they appear to differ little from the tactile bristles, except in size.

The palps

(*d*) *The palpal organ*, which is situated at the tip of the palp on the ventral surface, has been briefly described in *Ixodes ricinus* by Samson (1909) and in *I. melicola* by Schulze (1941). The organ consists of a beautifully arranged cluster of sensilla of the 'chemoreceptor' type, inset on a small area of cuticle representing the rudimentary 4th palpal article (Figs. 25 B, 26 A). Like the hairs of the posterior capsule these sensilla are often slightly curved, have rounded tips and thin delicate walls. A section of the palpal organ, showing the underlying groups of sense cells is given in Fig. 26 B.

Besides the palpal organ itself the palps bear a number of tactile bristles and short, straight hairs.

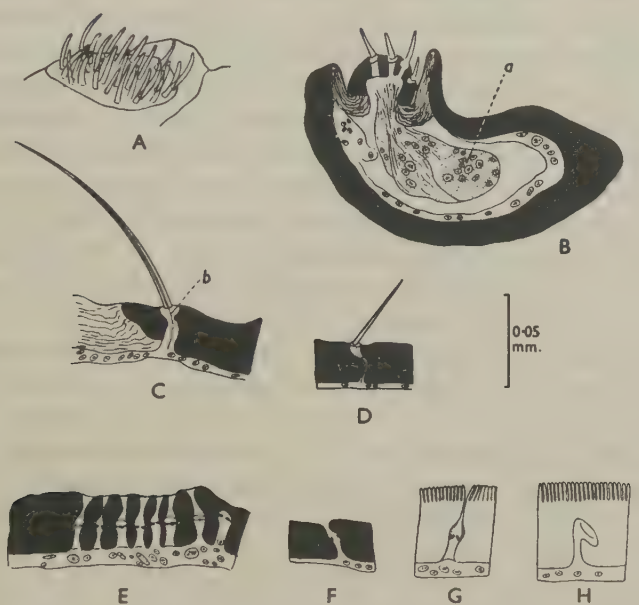

Fig. 26. A, palpal organ; B, transverse section of the tip of a palp to show the palpal organ; C, a tactile bristle; D, a temperature sensillum; E, longitudinal section through the porose area; F, G, ducts of dermal glands from scutum and alloscutum respectively; H, a defective duct from the alloscutum; *a*, sense cells; *b*, terminal filament.

Sense organs elsewhere on the body

The remaining three pairs of legs do not, of course, bear any structure comparable with Haller's organ, but otherwise the types of sensillum encountered, and their distribution, are very similar. The ventral surfaces of all the legs are freely clothed with long, slender tactile bristles while the short, straight hairs are again present on the dorsal and lateral aspects of the leg. In one female sixty-two of the latter were counted on the left foreleg, forty-eight on the second, forty-five on the third and thirty-seven on the fourth. The progressive decline in the numbers of this type of sensillum from the 1st to 4th pair of legs seems to be a constant feature. The rest

of the body bears a number of long tactile bristles, but is almost entirely devoid of the smaller hairs.

A careful search has failed to reveal any sensilla resembling the campaniform or chordotonal organs of insects. In the mite *Erythraeus*, long, spindle-shaped sense cells are attached to the intersegmental membrane of the legs and may serve as proprioceptors during leg movements (Gossel, 1935). If this type of receptor were present in *Ixodes*, the most likely position would appear to be on the membrane between the 2nd and 3rd segments of the foreleg, since movement takes place most freely about this articulation. No such sensilla could be distinguished, however, in sections of the leg.

Some of the many structures in ticks which have been claimed as sensory must also be mentioned. Schulze (1942*a*), in a recent morphological examination of the dermal sense organs in ticks, distinguishes two groups of sensilla: (i) trichoid sensilla, and (ii) 'krobylophores' or 'Schopforgane'. The first category includes the 'sensilla auriforme', and the second, three types of organs described as the sensilla 'sagittiformia', 'hastiformia' and 'laterniformia'. All these so-called sense organs are duct-like structures, often of complicated shape, which penetrate the cuticle. The 'sensillum auriforme', which Schulze believes to be a proprioceptor responding to strains set up in the cuticle, alone fails to penetrate to the surface of the cuticle and ends in an expanded knob within the endocuticle. The 'krobylophore organs' are claimed as a new type of receptor responding to both chemical and mechanical stimulation; in addition they are also said to be secretory. In the larger organs (sensilla sagittiformia) Schulze describes a movable tufted ending ('Endstift') which projects freely into the cuticular canal of the organ; he is of the opinion that a similar element is also present in the smaller 'sensilla hastiformia', although this could not be distinguished with certainty. In *Dermacentor andersoni* Douglas (1943) regards the equivalent of the 'sensilla hastiformia' of Schulze as integumentary glands without sensory innervation, but remarks that they appear to resemble chemoreceptors rather than glandular organs. Sensory pits (equivalent to Schulze's 'sensilla sagittiformia') are also present in *Dermacentor*. They consist of solid cuticular bodies shaped like a truncate cone, suspended by a thin membrane in a deep depression of the integument; the cone-like body is attached to a sensory cell.

The small duct-like structures appear to be present in the integument of all species of ixodid ticks as well as on the stigmal plate. Earlier opinion, however, was divided in regarding them as sense organs or the ducts of integumentary glands. Williams (1905) refers to them as dermal glands in *Boophilus annulatus*; Bonnet (1907) and Nordenskiöld (1908) consider the structures in *Ixodes ricinus* to be glandular, the latter comparing them to the defence glands of caterpillars. On the other hand, Nuttall *et al.* (1908*b*) regard the ducts on the stigmal plate of *Haemaphysalis* as sense organs, and Falke (1931) refers to them in *Ixodes* as 'Champagnerpropforganen'.

Much of the confusion has arisen owing to the fact that most authors have examined the structures in unfed or engorged ticks but not in both. The development of the cells associated with two types of dermal structure has recently been

described by the author in *Dermacentor andersoni* and *Ixodes ricinus* (Lees, 1947). The two types of integumental gland present in *Dermacentor*, corresponding to the 'pit organs' and 'integumental glands' of Douglas (1943), were both regarded provisionally as dermal glands and were referred to as types *A* and *B*. Only dermal glands of type *B* are present in *Ixodes*. In the unfed tick, the cells at the base of the ducts are hardly distinguishable from the other epidermal cells, but when the tick engorges they hypertrophy enormously and finally degenerate, yielding a greasy yellow end-product which may pass up the duct into the cuticle surface. This is the 'waterproofing secretion' referred to by Schulze (1942*a*). Reasons have been given for doubting this interpretation however (Lees, 1947).

The cuticular ducts in *Ixodes ricinus* (the 'sensilla hastiformia' of Schulze, 1942*a*) were re-examined in sections of the cuticle, but in spite of careful search no trace of any internal motile element could be detected. In the unfed female each duct is essentially an open canal with a wide base into which cytoplasm of the epidermal cell projects (Fig. 26 G). The cytoplasm extends only half-way up the duct to a point where the walls are somewhat thickened and expanded in diameter; the duct is then continued to the surface as a fine, thin-walled tube. The associated nuclei lie outside the pore in the epidermis and there appears to be no innervation. Large numbers of ducts are present on the hard, sclerotized parts of the body, such as the legs and scutum, as well as on the distensible regions (Fig. 26 F, G). A certain proportion are always malformed: quite commonly the basal canal is normal, but the narrow outer pore is missing, the gland therefore failing to reach the exterior (Fig. 26 H). It is noteworthy that some of these defective ducts closely resemble the examples of the 'sensilla auriforme' described by Schulze (1942*a*, e.g. Fig. 21*b* and *g*).

The *porose areas* in ticks have also been commonly regarded as sense organs of unknown function (Samson, 1909; Falke, 1931; Schulze, 1942*b*; Douglas, 1943). The areas are present in the female tick only and consist of two oval cribriform regions situated on the posterior dorsal surface of the basis capituli. In section (Fig. 26 E) the porose areas are seen to consist of a compact group of ducts, each of which closely resembles the more scattered ducts from the hard regions of the integument. There are many references in the literature to the presence of sense cells inside the basal portions of the ducts (Bonnet, 1907; Douglas, 1943); and Douglas also describes the innervation of the porose areas in *Dermacentor* by the paired oculaporosa nerves arising from the lateral cerebral ganglion. I have been unable to satisfy myself of the presence of sense cells or of innervation in *Ixodes*, and prefer to regard the areas provisionally as groupings of tegumental ducts.

The dorsal *foveae*, which are morphologically very similar to the porose areas, and which have likewise been regarded as sense organs (Schulze, 1942*b*; Douglas, 1943), are absent in *Ixodes*.

THE LOCATION OF THE SENSES

Tactile stimuli

The long tactile bristles on the legs are undoubtedly responsible for the perception of vibrations, as is witnessed by the instant questing response of ticks resting on a glass rod. The long recumbent bristles of the tarsus are well placed for such a function for, with the forelegs folded in repose, their tips rest lightly against the substratum. And we have already noted that on the distal articles these sensilla are confined to the ventral surface of the leg.

Tactile stimulation is probably also of importance in the typical behaviour on the short vertical rod. The recurrent upward-turning movements near the tip are shown only by ticks which are questing as they walk. The course pursued by an unfed nymph whose forelegs have been amputated, or that of a nymph climbing with the assistance of the forelegs, is entirely different (Fig. 27 A, B). In both cases the tick walks steadily up and down the rod without executing the normal upward-turning movements near the tip. In the first instance, the response probably fails because the tactile sensilla of the forelegs are missing, and in the second because the forelegs are being used for locomotion, rather than for carrying out the usual exploratory investigation of the tip (p. 148). No proprioceptors which would influence the vertical movements directly have been discovered.

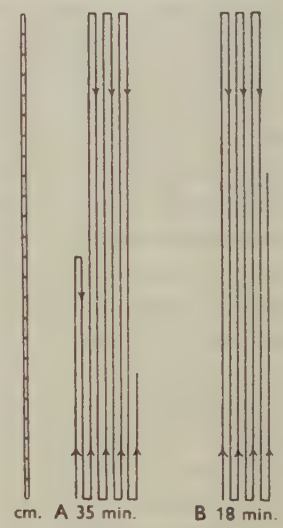

Fig. 27. Tracks of ticks climbing a vertical rod. A, unfed nymph with forelegs amputated; B, unfed nymph walking continuously with the aid of the forelegs (not questing).

Temperature

Questing ticks encountering warm or humid air or an unfavourable odour wave their forelegs vigorously before orienting; and it is clear from such movements that the forelegs bear sensilla sensitive to temperature, humidity and smell. Obviously Haller's organ is involved in these reactions. In order to identify the sensilla, the behaviour of unfed females was observed individually after Haller's organ had been eliminated. Each tick was tested successively: (i) on a horizontal rod walking up a steep humidity gradient extending from 34 to 100% R.H.; (ii) with sheep wool at room temperature (about 21° C.); (iii) with a warm odourless tube at 37° C.; and (iv) with a warm tube in the presence of sheep wool. A positive response to humidity is shown if the tick turns back on entering the high humidity; a response to temperature by an avoidance of, or an attraction to, the warm odourless tube; and a positive response to smell (in some individuals) by the repellency of the warm odourless tube and its attractiveness in the presence of wool.

An attempt to locate the olfactory sensilla by using the avoiding response to citronella was abandoned as ticks were occasionally repelled at close quarters even

when Haller's organ (which further work showed to be the olfactory receptor) was out of action. The reason for this is not known, but it is possible that such powerful and unfavourable odours may stimulate sensilla other than the true olfactory organs.

To eliminate Haller's organ a spot of quick-drying cellulose paint was applied to the organ so as to cover the anterior and posterior bristle groups while leaving the rest of the tarsus free. At first the ticks made persistent, and sometimes successful, attempts to remove the covering: in these typical reflex cleaning movements, which have been described by Samson (1909), the tarsus of the foreleg is repeatedly drawn through the elbow of the tibia and tarsus of the second pair of legs and is released with a peculiar flicking motion. This action which is often performed by normal ticks, will certainly have the effect of freeing Haller's organ from contaminating matter. After applying the paint the ticks were always allowed 24 hr. in damp air for recovery.

The reactions of ten hungry females to these stimuli are given in Table 8 A. In this section on the temperature response, we are only concerned with the results of tests (iii) and (iv). We see that nine ticks out of ten were repelled by the warm tube

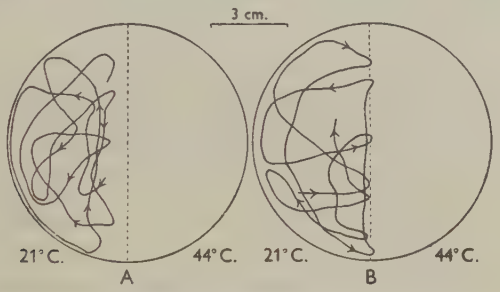

Fig. 28. Track of unfed female exposed to alternative temperatures of 21 and 44° C. A, forelegs intact; B, the same after amputation of both forelegs.

and were still repelled after Haller's organ was covered. Yet the movements of the forelegs showed clearly that the temperature difference was still being perceived by sensilla located on the forelegs. One female (index no. 7) which was attracted and not repelled by the warm odourless tube, yielded a result of particular interest, for without Haller's organ this tick was still attracted by this stimulus. From these findings it is clear that the temperature receptors are not borne on Haller's organ. Now, besides the long tactile bristles, the only remaining type of sensillum present on the forelegs are the short, straight hairs scattered on the dorsal and lateral aspects of the leg. These are almost certainly the temperature receptors. Situated exclusively on the dorsal and lateral aspects of the foreleg these sensilla are more advantageously placed for perceiving minute heat differences in the surrounding air (their normal function) than for detecting temperature differences on the substratum.

As sensilla of this type are present, though in smaller numbers, on the other legs and on the palps, the response to temperature should not entirely disappear if the thermoreceptors of the forelegs are eliminated. The type of response illustrated in Fig. 28 where the tracks of a single unfed female in an alternative chamber with temperature differences of 21 and 44° C. are followed, shows this assumption to be

correct. With the forelegs intact this tick only approached to within 1 cm. of the boundary before executing a successful avoiding reaction (Fig. 28 A). After the forelegs had been amputated the taxis still persists (Fig. 28 B), but the tick crosses to the centre of the chamber and turns back only when contact is established with the floor of the warm side. In *Pediculus* also, the temperature receptors are not confined exclusively to the anterior regions of the body (Wigglesworth, 1941). Since there seem to be no anatomical differences between the temperature sensilla on the forelegs of the tick and those borne elsewhere, the failure to locate a distant source of warm air is evidently associated with the mobility of the forelegs: by their aid the tick can obtain an accurate picture of the temperature gradient without further exploration. In the absence of distance perception the attractive qualities of warm air also disappear. Unfed ticks of all stages when lacking their forelegs are always repelled, and are never attracted by a warm tube.

Sensilla responsible for the temperature sense have been identified in few insects. In the blood-sucking bug *Rhodnius* two types of hairs, borne on the antennae, are regarded as sensiiive to temperature (Wigglesworth & Gillett, 1934*a*); the larger are devoid of sockets and, like the tick thermosensilla, are thick-walled and taper to fine points.

Humidity and smell—Haller's organ

Referring to Table 8 A, we see that seven out of the ten ticks were at first repelled by a high humidity, but after Haller's organ had been covered all of these became indifferent and walked unhesitatingly into the damp air. Evidently Haller's organ is concerned with the humidity response.

Table 8. *The function of Haller's organ*

A. The reactions of ten hungry female ticks to a high humidity, to a warm odourless tube, to a warm tube in the presence of sheep wool and to wool alone, before and after complete elimination of Haller's organ. o = indifferent; + = attracted; − = repelled. B. The reactions of ten females to humidity and smell after partially covering Haller's organ.

A

No. of ticks	Index nos.	Haller's organ uncovered				Haller's organ covered			
		100% R.H.	37° C. odourless	37° C. wool	21° C. wool	100% R.H.	37° C. odourless	37° C. wool	21° C wool
6	1–6	−	−	+	o	o	−	−	o
1	7	−	+	+	o	o	+	+	o
2	8–9	o	−	+	o	o	−	−	o
1	10	o	−	+	+	o	−	−	o

B

No. of ticks	Organ covered	100% R.H.		37° C. wool		37° C.	
		No. repelled	No. indifferent	No. attracted	No. repelled	No. attracted	No. repelled
5	Anterior pit	o	5	5	o	o	5
5	Posterior capsule	3	2	o	5	o	5

As regards the response to smell, one tick was attracted by temperature alone and so is without significance in identifying the olfactory receptors. Nine ticks, on the other hand, were repelled by the warm tube alone, but were strongly attracted when sheep wool and the warm tube were presented together. After covering Haller's organ these females ceased to be attracted when both stimuli were presented and all refused to approach the tube. In other words, the response is the same as to temperature alone. Only one tick (index no. 10) was weakly attracted by wool at 21° C.; and this response disappeared after Haller's organ had been put out of action. Haller's organ then must also contain the olfactory sensilla.

Since Haller's organ is composite and includes two, morphologically distinct groups of sensilla in the anterior pit and posterior capsule, it seems probable that one organ is the hygroreceptor and the other the olfactory organ. Owing to their minute size and the proximity of pit and capsule the identification of the receptors proved to be difficult. An attempt was made to settle this question by covering one-half of Haller's organ and leaving the other free. As even slight exposures to ether or chloroform resulted in permanent injury, this operation was performed without anaesthesia. In the technique finally adopted the body of the tick with the forelegs outstretched was held down with the forefinger whilst the pulvillus of each foreleg was firmly embedded in a small pad of plasticine. The cellulose paint was then applied under the highest power of the binocular on the head of a fine entomological pin. The ticks were always allowed the full 24 hr. for recovery after this treatment. The females used in these experiments were previously selected for their strong response to humidity and for their consistent avoidance of the warm tube and attraction to wool in the presence of the warm tube.

Table 8 B shows that of the five females with the pit covered, five were attracted by smell in the presence of warm air while none responded to humidity. On the other hand, all the ticks with the capsule covered were repelled by smell in the presence of warm air, while three still responded to humidity. Although not entirely conclusive, these results suggest that the sensilla of the pit are sensitive to humidity and those of the capsule to smell. This identification is not surprising, for the general appearance and thin walls of the capsule sensilla clearly indicate that they are chemoreceptors; indeed, it had previously been assumed that their function was olfactory (e.g. Schulze, 1941).

Little is known of the humidity receptors in arthropods. In the mealworm beetle, *Tenebrio*, they have been identified as peg and pit-peg organs on the antenna (Pielou, 1940); and in *Pediculus* as the tuft organs (Wigglesworth, 1941). In both insects, the sensilla are believed to act as hygrometers, the perception of humidity changes depending on the hygroscopic properties of the cuticle. It has been pointed out that the greater sensitivity of the response at the higher humidity levels may be accounted for by the ability of the hygroscopic material to absorb more water in moist than in dry air for a given increase in relative humidity. The same argument would apply with equal force to *Ixodes*. The sensilla of the anterior pit, with their thin delicate tips, seem well adapted for perceiving changes in humidity in this way. It is possible, for example, that slight changes in the curvature of the

hair may stimulate the nerve endings. However, no such differences could be detected with certainty under the microscope when dry and then moist air was passed over an isolated foreleg.

The peg and pit-peg organs in *Tenebrio* are regarded both as olfactory and humidity receptors (Pielou, 1940). And in spiders the 'tarsal organ', a deep pit enclosing a group of thin-walled sensilla, has also been shown to be responsible both for the perception of odours and for a response to drinking water when the tarsus comes into contact with any moisture (Blumenthal, 1935). Although the tarsal organ and Haller's organ are often held to be homologous (Schulze, 1941) the resemblance, morphological and physiological, may perhaps be rather remote. Thus all the sensilla of the tarsal organ appear to be of the same peg-like character and there is no structure in the spider corresponding to the anterior pit. The senses of smell and humidity seem to be quite distinct in the tick, as indeed they are in the louse (Wigglesworth, 1941).

As the directed response to humidity is dependent on the sensory stimulation of Haller's organ, one may inquire whether the undirected reaction, or kinesis, which comes into operation after desiccation, does not also rest on the perception of humidity differences by the sense organs. To determine whether any such sensilla are present on the forelegs, which in fact bear the main sensory equipment, a series of tests was carried through with ten unfed female ticks both before and after the forelegs had been amputated. The ticks, which were observed individually in small activity chambers at 20° C., were subjected to the following experimental conditions: they were either active to start with or at rest with their legs folded; their water balance was either normal (previously in saturated air for at least 2 days) or depleted by desiccation; and the atmosphere in the chamber was either dry (0% R.H.) or moist (100% R.H.). To stimulate activity the females were picked up with the forceps and laid down on their backs in the centre of the arena. The state of desiccation, critical for the operation of the kinesis, was obtained by observing the ticks in dry air until spontaneous movements began. For observing desiccated but inactive ticks in dry air, the desiccated females were first exposed to saturated air for the brief period required to arrest their movements (see p. 159). The average duration of the initial period of activity or inactivity is recorded in Table 9.

As was expected, the normal ticks come to rest relatively slowly in dry or moist air, whereas, after desiccation, their movements are very rapidly arrested in moist air; or are provoked in dry air. Ticks deprived of their forelegs, and therefore of Haller's organ and a large proportion of the temperature sensilla, are far more lethargic and less responsive than normal animals. The condition is somewhat the same as in *Rhodnius* when this insect is lacking the antennae (Wigglesworth & Gillett, 1934*a*). The tick remains immobile almost indefinitely but can be aroused (sometimes with difficulty) by pinching the body with the forceps or by warming in the hand. The tendency for the movements to be arrested in dry or moist air is very pronounced, even when the water balance is normal. Thus the average duration of locomotion in moist air was only 8 min. as compared with 102 min. when the legs were intact (Table 9). Nevertheless, in spite of this tendency, we observe that the

kinesis still operates after desiccation, for the desiccated ticks were aroused from inactivity after only 6 min. of continuing desiccation.

These results show clearly that the kinetic response to humidity persists after most of the sense organs have been eliminated. Although some temperature sensilla, and possibly other unidentified receptors, remain, it is considered probable that no sense organs are concerned. The possible mechanisms of the reaction have already been discussed (p. 159).

Table 9. *The effect of amputation of the forelegs on average time taken for ten unfed female ticks to come to rest or to begin walking in moist or dry air*

	Minutes $\pm \sigma_M$
Forelegs intact	
Normal water content:	
(i) Active in 100% R.H.; inactive after	102 ± 24
(ii) Active in 0% R.H.; inactive after	80 ± 14
Desiccated:	
(i) Active in 100% R.H.; inactive after	6 ± 1·3
(ii) Inactive in 0% R.H.; active after	10 ± 2·7
Forelegs amputated	
Normal water content:	
(i) Active in 100% R.H.; inactive after	8 ± 2·7
(ii) Active in 0% R.H.; inactive after	16 ± 8·2
Desiccated:	
(i) Active in 100% R.H.; inactive after	8 ± 1·8
(ii) Inactive in 0% R.H.; active after	6 ± 1·5

Function of the palpal organ

It has so far been assumed that the palpal organ is not concerned with the distance perception of smell, temperature or humidity. This has been confirmed by observing the behaviour of ten unfed female ticks, first with the palpal organ free, then with the organ covered with a coating of cellulose paint. The reactions to a high humidity, to a warm tube in the presence or absence of sheep wool, to sheep wool at 20° C., and to the odour of citronella, are given in Table 10. Of eight ticks avoiding the high humidity, seven behaved similarly when the palpal organ was out of action.

Table 10. *The reactions of ten hungry female ticks to various stimuli before and after the elimination of the palpal organ*

o = indifferent; + = attracted; − = repelled.

No. of ticks	Palpal organ uncovered					Palpal organ covered				
	100% R.H.	37° C. odourless	37° C. wool	20° C. wool	citronella	100% R.H.	37° C. odourless	37° C. wool	20° C. wool	citronella
3	−	−	+	o	−	−	−	+	o	−
1	−	−	−	o	−	−	−	−	o	−
1	−	+	+	o	−	−	−	+	o	−
1	o	−	−	o	−	o	−	+	+	−
1	o	+	+	o	−	o	−	−	o	−
2	−	−	+	o	−	−	−	−	o	−
1	−	−	+	o	−	o	−	−	o	−

Eight ticks which were repelled by warmth alone were also repelled subsequently, and of eight ticks to be attracted by warmth in the presence of wool, five were attracted when the organ was covered. All the ticks were repelled by the odour of citronella with or without the palpal organ. Evidently the reactions to these stimuli remain substantially unchanged so long as Haller's organ and the forelegs are retained.

Although the palpal organ is not concerned with the distance perception of chemical stimuli, the sensilla are clearly of the chemoreceptor type. The following observations on the behaviour of ticks approaching a patch of a repellent such as citronella, suggests that the organ functions as a contact chemoreceptor.

A circular area 3 cm. in diameter was marked out in the centre of a sheet of filter paper by dipping the mouth of a specimen tube into molten paraffin and applying it hot to the paper. A drop of citronella placed in the centre was thus prevented from spreading further than the waxed boundary. Typical tracks obtained with normal and operated unfed females are given in Fig. 29. The operations involved the amputation of the forelegs at the base and cutting through the tips of the palps to eliminate the palpal organ.

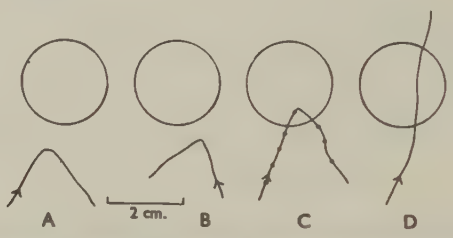

Fig. 29. Tracks of four unfed female ticks approaching a patch of citronella. A, normal tick; B, forelegs intact but tips of palps cut through; C, forelegs amputated but palpal organs intact. Positions where the tips of the palps touched the substratum are indicated by dots; D, forelegs and palpal organs amputated.

When Haller's organ is retained, normal ticks and those without the palpal organ still turn away sharply at a distance of about 1 cm. from the edge of the patch (Fig. 29 A, B). On the other hand, ticks lacking the forelegs but retaining the palpal organ advance right on to the patch before turning aside sharply (Fig. 29 C). Now as the tick walks, the tips of the palps are brought into contact with the substratum at each forward step. As this figure indicates, the avoiding response ensues only when close contact with the citronella has been established. Finally, when both the palpal and Haller's organs are missing, the tick advances across the patch without hesitation (Fig. 29 D).

The palps, unlike the forelegs, clearly play no part in the perception and localization of distant chemical stimuli and are never moved except for cleaning and other special operations. Although the stimulation of the palpal organs under experimental conditions may lead to an avoiding response, it is probable that they are rarely, if ever, used for orientation in nature. Their function as contact chemoreceptors undoubtedly comes into play during the preparatory period on the host,

before attachment takes place. This is discussed in a following section. A similar condition has been described in the blowfly *Cynomyia* by Frings (1941). In this insect sensilla on the antennae function as distance receptors and are involved chiefly in chemotactic orientation. Certain sensilla on the labellum are also olfactory, but are used in the perception of the food odours rather than in orientation and, in addition, there may be true gustatory receptors on the labellum. Whether the palpal organ of the tick is regarded as a contact chemoreceptor, as a gustatory organ or as an olfactory receptor with an exceptionally high threshold of stimulation, perhaps becomes a matter of definition only. The first interpretation is probably the most appropriate if, in defining taste, we refer only to those classes of substances to which the human tongue is sensitive.

Light

Not all the regions of the integument are equally sensitive to stimulation by light. Thus orienting movements are most rapid if the engorged tick encounters a horizontal beam of light head-on. And the correct orientation is clearly maintained by keeping the capitulum shaded by the convex body while the posterior surface of the dorsum remains brilliantly illuminated. One infers from this behaviour that the anterior regions of the body are particularly responsive to increased light intensities. It has not been possible, nevertheless, to delimit the receptor areas with any precision. Engorged female ticks with the anterior half of the body (including the capitulum, porose areas and scutum) covered with a thick coating of opaque cellulose paint still retreated from an intense horizontal light. It may be that the legs, which remained uncovered in these experiments, are equally sensitive to dermal stimulation.

ATTACHMENT TO THE HOST

After transferring itself to a mammalian host, a hungry tick becomes intensely active, walking rapidly with the aid of the forelegs and clinging on tenaciously. The movements are entirely random, but on a large host, such as the sheep, the final position of attachment is characteristic, depending on the point at which the tick climbs on (Milne, 1947*b*). Most of the ticks are picked up on the head while the animal is grazing and are found attached in this region. Some climb on to the legs and walking upwards, attach in the axilla or groin. A tick is often found attached to the tip of the tail when this drags over the ground. The long wool of the flanks brushes many ticks from the vegetation and a few of these finally attach in the 'partings', but many others, which are seen struggling over the surface of the fleece, undoubtedly become desiccated and fail to find a suitable attachment site. Over the head region of the sheep the distribution of the different developmental stages is reflected in a rough zonation, with larvae predominating on the forepart of the nose and cheeks, nymphs further back on the bridge of the nose and under the jaw and female ticks predominating round the ears and in the short hair of the neck. The larvae and nymphs easily penetrate between the closely set hairs on the face, while the larger females tend to walk over the hairs and attach further back.

The sheep tick, like other species of Ixodidae, requires a preliminary period of sensory stimulation on the host before attachment takes place. Under experimental

conditions attachment is greatly influenced by the state of hunger, as the following example shows. Batches of ten female ticks which had moulted 7 months and 6 weeks previously were placed separately in small capsules secured to a shaven patch on the flank of a sheep. Of the older ticks, five had attached after 1 hr. on the host, nine after 4 hr. and ten after 24 hr. None of the younger ticks had attached after 4 hr. and only four and six after 24 hr. and 40 hr. respectively. Five ticks were still walking round the capsule after 24 hr. on the sheep; and after 40 hr. four of these had succumbed to desiccation without having attached.

The act of attachment takes place abruptly. Without warning the tick draws back the palps slightly and, appearing to 'stand on its head', pushes the capitulum through the hairs to the skin. The insertion of the hypostome and chelicerae seems to take place gradually, but the mouthparts are usually fully inserted after 10 min.

Stimuli inducing attachment and the role of the sense organs

Hindle & Merriman (1912), in experiments with *Argas persicus*, recorded that ticks deprived of their forelegs would feed through an artificial membrane of rat diaphragm on 0·8% sodium chloride or on gelatine solutions kept at a temperature of about 42° C., while normal ticks always failed to attach and feed. This is considered as additional evidence of the olfactory function of Haller's organ. Although the argument is not fully developed, these authors seem to imply that in attachment to a normal host, smell and temperature are both important stimuli; that a warm artificial membrane is repellent to a normal tick, but that in the absence of Haller's organ, the temperature stimulus alone can provoke attachment. Totze (1933) described numerous feeding experiments with *Ixodes ricinus* from which he drew somewhat similar conclusions. The ticks would not normally feed through a membrane of diaphragm or cellophane but they readily attached and fed on warm defibrinated blood when Haller's organ had been amputated. Ticks lacking the forelegs could also be induced to feed through a membrane on other media such as solutions of gelatine, sodium citrate or methylene-blue. It is stated that by raising the temperature of the blood, the period of engorgement in the female can be reduced from 8 to about 3 days. After this blood meal, the ticks are described as 'mehr oder weniger vollgesogen'; this appears to be the only statement in the literature to the effect that ixodid ticks can be induced to feed to near repletion through a membrane. Regarding the function of the sense organs in attachment, Totze asserted that nymphs or female ticks lacking the forelegs fail to attach if the hindlegs are also amputated, while normal attachments to the warm artificial membrane take place if either the 2nd or the 3rd pair of legs are lacking. He postulates two types of thermoreceptors, namely, contact receptors on the hindlegs in the absence of which no attachment can take place, and distance receptors generally distributed over the rest of the body. More recently, membrane feeding of argasid ticks (in which engorgement is rapid) has sometimes been utilized with other ends in view. *Ornithodorus moubata* can be fed on blood enriched or deficient in chloride (Boné, 1943); but attachments appear to take place most readily if a natural skin is used as a membrane and if Haller's organ is left intact.

Method

Certain of these experiments with *Ixodes ricinus* have been repeated with the object of determining the function of the sense organs in the attachment response. The following sites were provided for attachment: (i) the living sheep, (ii) freshly prepared lambskin membranes, and (iii) membranes prepared from the diaphragm of the rabbit. On the sheep, the ticks were placed in capsules 3 cm. in diameter, securely fastened to the flank by means of a beeswax-resin mixture (Burtt, 1945). The membrane-holders were 3 × 1 in. glass tubes open at both ends. The lambskins or diaphragm were stretched over one end and secured. After drying out, the diaphragm, which appeared to be completely odourless, was slightly greased on the outer side to reduce the excessive transpiration of water. The holders, which were inserted through corks, were held in an outer vessel containing freshly prepared citrated human blood to a depth of about 0·5 cm. The undersurface of the membranes were always completely immersed in blood. After placing the ticks inside the membrane-holder the other end of the tube was closed with a plug of cotton-wool. The membranes were either kept at room temperature (23–24° C.) or at 37° C. To provide the latter temperature, the whole apparatus was at first allowed to stand on a warm plate. Later, when it was found that attachments took place readily in the absence of a temperature gradient from the membrane surface, the apparatus was placed in an incubator at 37° C.

Ten hungry female ticks were used in each experiment. The operations, which were performed without anaesthesia, included amputation of the forelegs and hind-legs at the base, cutting through the tips of the palps to eliminate the palpal organ and combinations of these operations. Provided no blood was lost, nearly every tick afterwards survived normally. Attachments were counted after 1, 4 and 24 hr. Any ticks which had failed to attach by 24 hr. had always succumbed to desiccation.

Results

We may consider first the results with normal ticks (Table 11). On the living sheep, nine out of ten females attached, and with the lambskin at 37° C. (factors smell and temperature present) seven attached successfully. On the other hand, with the lambskin at 24° C. (smell present but temperature factor absent) and with the diaphragm at 37° C. (smell absent but temperature factor present) only one tick attached in each case. Finally, with the diaphragm at 23° C. (both factors absent) there were no attachments. Clearly, both thermal and 'chemical' stimuli are necessary for a successful response. In the absence of the former, the ticks display little interest in the attachment site and spend much time resting on the sides of the container; and in the absence of the latter, the ticks are intensely active, frequently crossing or recrossing the membrane without, however, ever stopping to probe.

Ticks which had attached to the lambskin were kept under observation for some days. During the first few hours after attachment they passed a little undigested blood through the anus as is usual in the engorging tick. However, there was practically no distention of the body and the ingestion of blood appeared to cease

after a few hours. Cessation of feeding may have been due partly to the unpalatability of the blood which, in spite of frequent changes, could not be kept in a fresh condition, and partly to the fact that there was always some leakage in the membrane around the point of insertion of the proboscis.

Since the warm lambskin membrane proved almost as attractive as the living sheep, the factors associated with the use of the apparatus which tend to discourage attachment cannot be of any considerable significance. In further tests it was thus legitimate to compare the behaviour on the living sheep (as supplying all the stimuli necessary for attachment) with the attachments to the diaphragm. As Table 11

Table 11. *The attachment responses of unfed female ticks*

Amputation	Attachment site	No. of ticks	No. attached after		
			1 hr.	4 hr.	24 hr.
None	Living sheep (flank)	10	5	8	9
	Sheep skin at 37° C.	10	5	5	7
	Sheep skin at 24° C.	10	0	1	1
	Diaphragm at 37° C.	10	0	0	1
	Diaphragm at 23° C.	10	0	0	0
Forelegs	Living sheep	10	6	6	6
	Diaphragm at 37° C.	10	0	0	1
Palpal organ	Living sheep	10	6	7	7
	Diaphragm at 37° C.	10	0	0	0
Forelegs and palpal organ	Living sheep	10	0	0	0
	Diaphragm at 37° C.	10	0	0	0
Hindlegs	Living sheep	10	5	10	10
	Diaphragm at 37° C.	10	0	0	0

shows, after amputation of the forelegs, six out of ten ticks successfully attached to the living sheep but only one to the diaphagm at 37° C. As we have seen, these individuals will lack the specific olfactory organ (the posterior capsule of Haller's organ) and some, but by no means all, of the thermoreceptors. The greater number of attachments to the living sheep are evidently connected with the perception by the palpal organ of the 'chemical factor' of the fleece which is absent from the diaphragm. Totze's claim (1933) that ticks deprived of Haller's organ readily attach to a warm, odourless membrane is not confirmed: in the present experiments, the females lacking the forelegs were as reluctant to attach to the warm diaphragm as were the unoperated animals. The diaphragm appeared to exert no repellent effect, the ticks being merely indifferent to it.

Seven ticks with forelegs intact, but without palpal organs, attached to the living sheep; none attached to the warm diaphragm. Finally, there were no attachments even to the living sheep among ticks lacking both the forelegs and the palpal organ. These results are consistent with the view that the stimuli inducing attachment are warmth, which is perceived by the temperature sensilla on the forelegs and elsewhere; and 'chemical factors' of the fleece, perceived as odour by Haller's organ or as a contact stimulus by the palpal organ chemoreceptors.

Totze's statement that the hindlegs bear contact temperature receptors of unique importance in attachment is incorrect. As Table 11 indicates, every tick lacking

only the hindlegs, attached normally to the sheep. This was to be expected, for although the hindlegs bear a few temperature sensilla, these are more sparsely distributed than on the other legs (p. 181). Totze attributed the failure of larvae to attach to his artificial membranes to the fact that the fourth pair of legs remains undeveloped in the larva. And he correlates this with the supposed larval preference for cold-blooded hosts. Since the ecology of the sheep tick first received detailed study (MacLeod, 1932) it has been realized that this frequently repeated statement is also erroneous, and that larvae are found in great numbers on mammalian hosts along with nymphs and female ticks. The fact that larvae and not nymphs or adults have been found attached to relatively unattractive cold-blooded hosts, such as lizards, is merely a consequence of the great preponderance of larvae in tick populations and has no bearing on 'host preference' (see Milne, 1948*a*).

ORIENTATION MECHANISMS

Certain reactions of the tick, notably the questing response to stimuli such as vibration, temperature and reduced light intensity, are postural and do not involve orientation in the first instance. The remaining sensory responses are locomotory; nevertheless, the mechanisms of orientation to different stimuli are not all alike. In gathering together the present results into a consistent scheme, the classification of Fraenkel & Gunn (1940), which draws a primary distinction between undirected and directed reactions (kinesis and taxes), has been followed.

Undirected reactions

In such reactions the body axis of the animal does not occupy a fixed position with respect to the stimulus. The postural questing is often a prelude to locomotion (e.g. in response to shading) or to locomotion followed by a directed orientation (e.g. in response to temperature). These are examples of orthokineses. Positive and negative orthokineses are also most important elements in the response of the desiccated tick to humidity, but are of no significance when the water balance is not depleted. The gregarious clustering of unfed larvae is an example of stereokinesis.

Klinokinesis, involving frequent changes in the direction of movement, accompanied by sensory adaptation, is very rarely seen in *Ixodes*. Fig. 30 illustrates two conditions under which such reactions were observed. In Fig. 30 A an unfed nymph is shown advancing up the flat floor of the temperature gradient apparatus. On failing to turn back the track becomes very convoluted at about 38° C. and only becomes straighter when the tick chances to enter cooler air. This is not the usual response to a high temperature: if the unfed adult tick avoids a warm tube at 43° C. the course followed after orientation is approximately straight. The reason is clearly that in the linear apparatus the gradient of about 1° C./cm. is so slight that the tick cannot perceive without further exploration whether it is rising or falling. When the gradient is steeper, this information is provided immediately by the testing movements of the forelegs, and the tick orients accordingly.

A second example of klinokinesis in response to the combined presence of certain attractive stimuli is shown in Fig. 30. In these experiments the arena consisted of

a sheet of filter paper with a circular area in the centre thoroughly rubbed beforehand with freshly cut sheep wool. The filter paper, supported round the edges, was held so that the central patch rested on the circular base of an inverted vessel of corresponding diameter which contained circulating water at 37° C. This, in effect, provided a localized source of warm air and of smell, in addition to contact chemical stimuli. Ticks approaching the edge of the patch oriented, and ran on to it. Some individuals then advanced straight across the patch and off the other side with only slight deviation from a straight course (Fig. 30 B); others, on reaching the further edge of the patch pursued a very tortuous course until they had regained contact with it (Fig. 30 C). Now klinokinesis was never observed in ticks which had oriented to, and were approaching, a warm tube with sheep wool wrapped round the base. If the tube is shifted slightly after it has first been located, the tick either reorients to the tube in its new position and approaches it afresh or abandons the orientation and walks off without further searching movements. It is clear that when stimuli of smell and temperature are perceived at all, the source can be located immediately

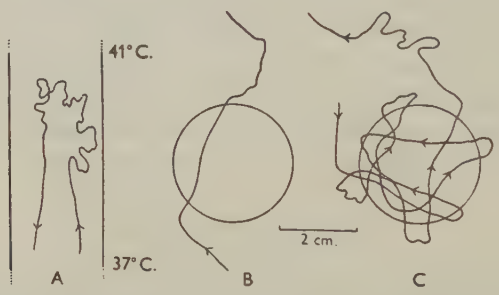

Fig. 30. Examples of klinokinesis. A, track of unfed nymph in the linear temperature gradient; B, C, tracks of ticks approaching a warmed patch of filter paper which has also been rubbed with fresh sheep wool; B, an unfed female; C, a male.

and accurately without random turning movements. This recalls the behaviour of *Rhodnius* (Wigglesworth & Gillett, 1934*a*) in thermal orientation rather than that of *Pediculus* (Wigglesworth, 1941). Probably the increased rate of change in direction after leaving the sheep-scented patch is associated particularly with the diminished stimulation of the palpal organ contact chemoreceptors.

In the figures published by Totze (1933) illustrating the behaviour of *Ixodes ricinus* in a gradient of temperature or smell (butyric acid) the tracks are shown as becoming more convoluted as the tick departs from the 'preferred' or 'optimal' zone. This has been interpreted as a klinokinesis (Fraenkel & Gunn, 1940). In the present observations undoubted examples of klinokinesis were confined to the above instances. No response of this type was ever observed to odours; and to temperature it is exceptional. Further, this mechanism certainly fails to occur in many other situations where the tick would be expected to experience slightly favourable or slightly unfavourable stimuli.

Directed reactions

The most characteristic reaction in orientation is a taxis. This may either be positive or negative, the tick orienting towards or away from the stimulus. Examples of the negative response to different stimuli include the avoiding responses shown to a high humidity, to warm air in the absence of a favourable smell (adults) or to citronella. The positive response is shown to warm air (nymphs and larvae) and to a favourable smell in the presence of warm air (all stages). Examples of the tracks have been given in Figs. 14 and 19. It is worth inquiring whether orientation is achieved by comparisons of intensity at successive moments (klinotaxis) or whether orientation follows from simultaneous comparisons carried out by sensilla borne bilaterally on the forelegs (tropotaxis). Wigglesworth & Gillett (1934*a*) have shown that the accuracy with which *Rhodnius* can locate a warm tube wrapped in a mouse skin is only slightly impaired if one antenna is amputated. When very near the tube, however, some of the bugs turned sharply towards the intact side and probed, thus suggesting that, while they are guided to the tube by klinotaxis, tropotaxis is of some importance in the final location of the stimulus.

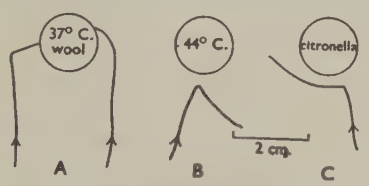

Fig. 31. Localization of sources of warm air and of odours by ticks (unfed females) lacking the left foreleg. A, consecutive tracks of the same tick approaching a warm tube with sheep wool; B, C, ticks approaching a warm odourless tube and a tube with citronella.

Unfed female ticks with one foreleg amputated were observed under the following conditions: (i) approaching the base of a tube at 37° C. in the presence of sheep wool; (ii) approaching a tube with filter paper moistened with citronella round the base; (iii) approaching an odourless tube at 44° C.; (iv) climbing a glass rod and exposed to alternative humidities at 34 and 100% R.H. Fig. 31 A shows the consecutive tracks of a female approaching the warm sheep-wool tube obliquely from the right or the left. This tick lacked the left foreleg, and, as the tracks indicate, the sensitivity was slightly greater on the uninjured side. However, the tick usually orients, runs up to the tube and climbs on to it in the usual manner. No tendency to turn towards the intact side was observed. Ticks with one foreleg can also locate and avoid a warm odourless tube, or a tube with citronella (Fig. 31 B, C), merely approaching rather more closely than usual. In avoiding the stimulus the ticks turn indifferently to the right or left. This point was tested particularly in relation to the humidity response. Three ticks out of eight which had their right forelegs amputated gave good avoiding responses of 100% R.H. on the vertical glass rod. Of twenty avoidances at the steepest part of the gradient, nine turns were made towards the intact side and 11 towards the operated side. One may conclude that the directed response is an example of klinotaxis, uncomplicated by any tropotactic element. The

successive comparisons of intensity are of course made by the waving movements of the forelegs and not by successive deviations of the body.

The directed response to light is presumably also a klinotaxis. No doubt successive comparisons of light intensity are made by the photoreceptive areas as chance deviations of the body axis occur. No regular side-to-side movements were detected.

Totze (1933) has contended the *Ixodes* seeks 'optimum' conditions of temperature, smell and light by avoiding lower and higher intensities of stimulation. Although no attempt has been made to repeat Totze's experiments under identical conditions, it is very doubtful whether this interpretation of the behaviour is correct. This concept cannot at least be reconciled with the reactions to temperature and smell described in the present paper.

THE SENSORY PHYSIOLOGY AND THE BEHAVIOUR IN THE NATURAL ENVIRONMENT

We may now attempt to assess the significance of sensory perceptions of *Ixodes* in terms of the stimuli encountered in the natural environment. Many of the statements made will be more fully elaborated in a later paper dealing with observations on the behaviour in the field (Lees & Milne, 1948).

After moulting, the unfed tick remains motionless for a variable period—sometimes months, sometimes as much as a year—near the roots of the vegetation. When the vegetation is of a character that favours the accumulation of partly decayed vegetable debris ('mat'), the tick usually takes up a position among the dead loosely intertwined grass stems and moss of the upper mat, only occasionally penetrating among the more tightly compacted material of the lower mat (Milne, 1948*b*). At the end of this period of quiescence, the tick climbs on to the vegetation above and remains there on the alert for a passing host. A variety of orienting reactions, all of which are of value in promoting survival or host-finding, are displayed in the natural environment. In attaining the goal of attaching to a suitable host, the tick experiences a series of sensory perceptions which may be grouped as follows: (i) those which bring the tick to a position where it is likely to encounter a host; (ii) those which give warning of the imminent approach of the host; and (iii) those which lead to attachment once the tick has transferred itself to the host.

The fully engorged tick withdraws the proboscis from the skin, drops to the ground and, crawling down into the vegetation layer, comes to rest. The movements of engorged female ticks after dropping have been described by Milne (1948*b*). They seem to be unable to reach cover which is more than 3 in. laterally from their point of arrival on the ground, and so, if they chance to fall on an expanse of short sward, they die of desiccation in a normal summer before ovipositing. If, on the other hand, they fall into deep vegetation they penetrate downwards as far as the upper mat with very little lateral movement and oviposit successfully. The reactions of engorged ticks are discussed under (iv).

(i) The movements of the unfed tick during the activity period are almost entirely in the vertical plane. The tick may walk up and down the lattice of tangled stems,

occasionally crossing from one to another when these lie adjacent, but it rarely moves far laterally. Experience has shown that the behaviour of a wild tick climbing the stout, smooth stem of a rush or grass is identical with that of a tick walking on a glass rod in the laboratory. The tick climbs steadily to the tip, quests, walks down a short distance, turns upwards again, repeating this performance several times before finally coming to rest near the tip of the stem. Detailed observations (Lees & Milne, 1948) have demonstrated that a very high proportion of 'active' ticks are confined to the top third of the vegetation layer; and a large proportion of these come to rest in the position shown in Fig. 1. It is clear that this response on a vertical rod-like object, in which, as we have seen, tactile perceptions undoubtedly figure largely, is of particular value in the natural environment. A position at the vegetation tips places the tick in a highly favourable situation for encountering the principal host, the sheep.

Humidity is also a very important factor. It is well known that the sheep tick is virtually confined to rough grazings supporting a thick covering of rank herbage which remains permanently damp near the roots. This distribution is certainly due to the high permeability of the cuticle to water, which renders the tick unable to withstand prolonged desiccation and thus to survive on more open-textured lowland pastures (Lees, 1947). Owing to the characteristics of the vegetation layer, one of the most constant features of the tick environment is the presence of a vertical humidity gradient extending from the mat to the vegetation tips. Actual conditions, although complex and variable, can be summarized by stating that during the day the humidity at the vegetation tips is relatively low and approximates to that of the macroclimate; while near the base of the vegetation layer, very high humidities prevail continuously. During the night the humidity near the tips rises and usually approaches saturation, but the gradient is rarely reversed (Milne, 1945, 1948*b*). Table 12 A records the average humidities of 'mat' (hygrometer inserted 1 cm. into upper mat) and 'tips' on a heavily tick-infested grazing; and Table 12 B some instances of the vertical gradient on selected dry (e.g. 9 April 1946) and wet (e.g. 11 April 1946) days in the same locality. These last measurements were made with Edney paper hygrometers suspended at various levels in the vegetation, namely, at the tips of the taller grasses and rushes (24 cm. above mat) and at the surface of the mat itself. As Table 12 B shows, the humidity gradient is steepest at the point where the longer stems penetrate the tangled growth of shorter grass. During the spring activity period, the tick is usually climbing on the long dead stems of last year's growth and not on vegetation that is actively transpiring, so it need not be assumed that the humidity the tick encounters on a stem will differ materially from that of the surrounding air.

Now we have recorded that unfed ticks with a normal water content often exhibit a strong negative hygrotaxis in a steep humidity gradient. Clearly, on leaving the moist microclimate at the roots after a period of quiescence, the tick will climb upwards without hindrance into drier air near the tips. If, however, after reaching the tip, the subsequent descent along the stem is unduly prolonged, exposure to rapidly increasing humidities will result once more in upward-turning. This type of

behaviour has occasionally been observed in the field. For example, a nymph which was seen climbing a vertical rush stem 26 cm. in height reached the tip, then walked downwards to a point 10 cm. above the mat where the stem was hidden from view by a dense tangle of fine grass. Here it turned upwards and again walked to the tip. This was repeated several times before the movements were finally arrested near the tip. Of course, in such a case it is impossible to decide whether upward-turning is a reaction to humidity or a delayed response to tactile stimulation at the tip. But the first possibility is strongly suggested by the fact that the tick descended fully 16 cm. from the tip on each occasion, and then turned upwards at nearly identical points on the stem at each subsequent descent. A humidity reaction of this kind will clearly assist the active tick to remain near the tips of the vegetation preparatory to coming to rest there. It would be unwise nevertheless, to overemphasize the

Table 12. *Temperature and humidity conditions in the vegetation layer at Upper Ewe Hill*

A. The average of forty-five observations made on separate days from 18 April 1941 to 30 June 1941. Data from Milne (1948*b*, and unpublished). B. Instances of the vertical humidity gradient, in % R.H., within the vegetation layer.

A

Vegetation type	Temperature (° C.)		Temperature difference between 'mat' and 'tips' (° C.)	Humidity, % R.H.		Humidity difference between 'mat' and 'tips' (% R.H.)
	'Mat'	Vegetation tips		'Mat'	Vegetation tips	
Grass	11·1	10·9	1·4	92	72	20
Bracken	12·8	14·0	1·8	92	69	23

B

Height above 'mat' surface (cm.)	Vegetation type			
	Grass and *Juncus* clumps			Bracken
	9 April 1946	11 April 1946	12 April 1946	12 April 1946
24	38	80	56	72
12	40	87	61	79
1	88	94	94	92

significance of this response which is only secondary to the much more important upward-turning reaction near the tips; this, of course, takes place in the absence of a humidity gradient.

After coming to rest near the vegetation tips, the tick is exposed to the normal atmospheric conditions and so will gradually lose water by evaporation. It has been shown that when the water balance becomes depleted locomotion is stimulated by continuing desiccation, whereas all movements are very rapidly arrested in damp air; and at the same time the hygrotaxis disappears. Clearly, under natural conditions the tick will be aroused before desiccation becomes critical and, walking down the stem, will come to rest in the moist microclimate near the roots. Once in this position it will take up water from the humid atmosphere and restore the normal water balance. The cycle of activity can then be resumed. Detailed observations of

the behaviour of marked ticks in the field has provided ample evidence of this type of behaviour (Lees & Milne, 1948). During a period of 'activity' ticks do not in fact remain continuously near the vegetation tips. After an unsuccessful period of waiting, they disappear from view, often reappearing a few days later near the tip of the same stem, from which indeed they have clearly never moved. It has been shown previously that unfed ticks reach a state of equilibrium in an atmosphere of about 90% R.H. and, when desiccated, are able to take up water through the cuticle from higher humidities (Lees, 1946). There is little doubt that the humidity of the microclimate near the roots remains continuously above this level: and that ticks behaving in this manner have replenished their water content while at the roots. Ticks at rest on exposed vegetation may be able to gain a little water at night time when humidities rise above the level at which the physiological process of water uptake can operate successfully (see Milne, 1945). Nevertheless, it appears that under the meteorological conditions usually prevailing, water loss during the day is not balanced by gain at night and they cannot thus remain at the tips continuously during the activity period without risk of serious desiccation.

Fluctuations of temperature are of undoubted significance in rousing the tick from quiescence at the roots and so initiating a phase of activity; but temperature differences within the vegetation lattice, although often present, are probably too small to influence orientation. The vertical temperature gradients on a typical rough grazing and their diurnal or seasonal trends have recently been examined by Milne (1948*b*) in relation to tick distribution. The records given in Table 12 A are derived from Milne's data and represent the averages of forty-five simultaneous observations of temperature within the mat and at the tips on different days during the spring activity period. On proceeding downwards into the vegetation layer the temperature at any particular moment may rise, remain unchanged or fall. If the direction of the gradient is ignored, the mean differences of temperature between mat and tips in grass and bracken plots were only 1·4 and 1·8° C. respectively (maximum 5° C.). Assuming the depth of the vegetation layer to be about 20 cm., the average gradient will be little more than 0·1° C./cm.

Light is also of little importance to the unfed tick. Under natural conditions in the upper mat ticks are probably exposed to very low intensities of illumination during the hours of daylight, and will be subjected to rapidly increasing light intensities as they walk upwards on to the unshaded vegetation. We have seen that the tick is never attracted by light, and may be repelled by directed illumination, particularly when newly moulted. As it ages, however, the tick soon becomes indifferent to such changes.

Among the physical factors influencing the behaviour of *Ixodes persulcatus* in its natural environment, gravity and humidity were considered by Mironov (1939) to be of most significance. In the Kama province of the U.S.S.R. this tick is not found on the vegetation at a height greater than 1–1·5 m. Mironov contends that the height the tick climbs is determined by the relative strength of two orienting responses, namely positive hygrotaxis (=a kinesis?) and negative geotaxis, which normally function in mutual opposition. Under usual British conditions the position

in the vegetation taken up by the active sheep tick is largely a reflexion of the depth of the vegetation layer itself, which is rarely more than 40 cm. over the treeless moorland grazings where the species is abundant. Larvae, nymphs and adults are all found at the same level near the vegetation tips and the turning response near the tips is probably the only orienting mechanism concerned in determining this distribution. If artificial conditions are created, the tick will sometimes climb so high that a meeting with a host on the ground would be impossible. This point was confirmed in the field by observing the behaviour of ticks on a length of fencing wire thrust vertically into the soil. A number of wild adult ticks were introduced into a small clump of grass at the base of the wire. In the course of the next few days most of these appeared on the wire and a certain proportion climbed to the tip and came to rest there, 6 ft. above ground-level. On the other hand, a tick will not continue indefinitely to climb a vertical object if it fails to reach a 'tip'. Nymphs, as we have seen (p. 150), seldom reach the tip of a 6 ft. rod. It is possible that under such conditions larvae, nymphs and adults will continue upwards to different heights before turning or coming to rest. There is in fact some evidence of zonation in situations where the vegetation layer is much deeper. Thus, according to Tambs-Lyche (1943), in certain swampy areas of Norway which support alders and other trees, adults of *I. ricinus* are found at a height where they could encounter only birds, cattle or horses. Nymphs are said to preponderate lower in the vegetation and larvae nearest the ground.

(ii) Certain sensory perceptions are of value to the tick in providing warning of the approach of a host. Mechanical disturbance of the vegetation is often sufficient to cause vigorous questing. A tick soon becomes adapted to the regular swaying movements of the vegetation in the wind but any abnormal movements of the stem usually cause instant alertness. The characteristic questing response which follows a sudden reduction in light intensity is shown very strikingly when ticks which are resting with their legs folded near the vegetation tips are shaded with the aid of a book held some distance to the leeward. The shadow cast by a large host, such as a sheep, would no doubt prove equally stimulating. Nevertheless, the readiness with which ticks attach themselves to a blanket dragged over the vegetation during the hours of darkness (Milne, 1947*a*) shows that this response is only subsidiary.

These stimuli have intensity but no direction and induce only a state of preparedness. The warmth and smell of the host's body, on the other hand, provide diffuse stimuli with the directional qualities needed for orientation; these stimuli are used in finally locating the host. The response is clearly seen when the observer offers his finger to ticks at rest near the vegetation tips. After questing, the tick orients and climbs eagerly on to the finger if it can be reached. The thermal and olfactory perceptions do not appear to be particularly acute (compared with *Rhodnius*, for example), but it must be remembered that the tick has comparatively little freedom of movement within the vegetation, and that the meeting of host and parasite, except at close quarters, is virtually dependent on the chance approach of the latter. The olfactory sense has been shown to lack species specificity, a fact which would be

suspected from the wide range of hosts the sheep tick is known to parasitize (Milne, 1948*a*).

Finally, the faculty possessed by the sheep tick, in common with other Ixodidae, for clinging to moving objects which brush against it, must be almost unrivalled among parasites. The significance of this tactile response can be judged by the success of blanket-dragging as a means of estimating relative ground populations (Milne, 1943), in spite of the absence of temperature and smell as attractive stimuli.

(iii) Having climbed on to the host the tick continues to experience temperature and olfactory stimuli as well as added stimulation as the palpal organ is brought into contact with the fleece or hair. The preliminary period of excitation when the tick is moving about actively on the host, eventually leads to attachment. The powers of the tick to remain in a small area providing all the factors necessary for attachment are limited in the extreme (p. 196). That it remains on the host until arriving at the final attachment site is due as much to the ability to cling closely to the host's skin as to any orienting movements.

(iv) The physical factors which influence the behaviour of the engorged tick after it has dropped off the host into the vegetation are likely to be relatively simple. Strong avoiding movements away from directed light filtering down from above result in downward penetration into the vegetation. The tendency of the tick to insinuate its body more deeply into spaces amongst the roots is no doubt assisted also by the need for contact. We have seen that the kinetic response to humidity persists in the engorged tick. It is quite possible that this is of considerable value during the summer drying out of the upper layers of the vegetation. If the moulting process has not begun, the engorged tick would be aroused by desiccation and would tend to settle down in the deeper and damper regions of the mat. Whether this ever occurs under natural conditions is not known.

The engorged sheep tick shows no well-defined response to gravity. In the related species *Ixodes canisuga*, a common parasite of the sheep dog, gravity reactions are of considerable significance. It is the usual practice in northern Britain to kennel the dogs in disused stables and outhouses, the stone walls of which are often intersected with deep crevices. The engorged ticks drop from the dogs to the floor, walk to the walls and begin climbing vertically upwards. In one kennel which was kept under observation a large number of ticks could always be found during the period of activity ascending the walls with plummet-like directness preparatory to entering a crevice and coming to rest.

SUMMARY

1. *Posture*

The unfed sheep tick when at rest adopts either a 'questing' attitude with the forelegs extended or an attitude of repose with the forelegs folded. While walking the hungry tick waves the forelegs in the manner of antennae.

2. *Sensory responses*

(*a*) *Gravity*. A tick walking on a 24 cm. vertical glass rod, which serves as a satisfactory model of natural grass or rush stems, usually climbs from the base to the tip, quests, then walks up and down near the tip before finally coming to rest there. This response involves a taxis (upward-turning near the tip) and a kinesis. Negative geotaxis may be of some significance, but reasons are given for supposing that this pattern of behaviour is partly a tactile response following arrival at the tip.

(*b*) *Humidity*. The humidity behaviour is greatly influenced by the physiological state. When their water balance is normal, unfed ticks avoid higher humidities but come to rest with equal readiness in moist or dry air. The avoiding response (taxis) disappears after desiccation and is replaced by a kinesis: the tick is then intensely active in dry air, but soon comes to rest in moist air. On remaining in damp air the water balance is restored by water uptake through the cuticle. The first response then reappears. The operation of the kinesis does not seem to depend on sensory stimulation. The taxis is weakly developed in normal engorged ticks, but the kinesis is again strongly manifested after desiccation.

(*c*) *Tactile responses*. Unfed ticks with their legs folded respond to vibrations by questing instantly: they readily cling to any object which brushes against them. The formation of densely packed larval clusters is a further response to contact stimulation.

(*d*) *Temperature*. Hungry ticks orienting to an odourless tube at 37° C. either approach it eagerly and climb on, or avoid it. This stimulus is usually attractive to larvae and nymphs, but repellent to adult ticks. The response is to a gradient of air temperature and not to radiant heat. Objects at any temperature higher than that of the surroundings may prove attractive, but avoiding responses are always elicited by temperatures higher than 42° C. In a linear gradient extending from 8 to 45° C. ticks aggregate rapidly in the coldest region after previous exposure to 25° C. Fewer cold-adapted ticks are trapped in this way; those remaining outside the coldest region show no definite 'preferendum' within the range 11–41° C.

(*e*) *Smell*. Below 20° C. ticks are indifferent to the odour of sheep wool alone; above 20° C. wool is slightly attractive. In the presence of a temperature gradient (wool wrapped round a tube at 37° C.) this odour becomes highly attractive. Thus, hungry adults which are repelled by temperature alone are attracted when both stimuli are presented together. They respond with equal vigour to the scents of dog, rabbit, cow and horse hair.

(*f*) *Light*. Unfed ticks at rest on a glass rod respond to a sudden fall in light intensity by questing (shading section). Engorged ticks are strongly photonegative; newly moulted ticks also avoid a directed illumination, but become indifferent as they age.

3. *Sense organs*

The following sensory structures are borne on the forelegs: (*a*) *Haller's organ*. This composite organ includes groups of sensilla in the anterior pit and posterior capsule which are morphologically distinct and have an independent innervation.

The sensilla of the anterior pit are the humidity receptors responsible for the taxis while the peg-like chemoreceptors of the capsule are olfactory. (*b*) *The temperature sensilla* are short, thick-walled hairs borne on the dorsal and lateral aspects of the leg. (*c*) *Tactile bristles* are restricted to the ventral surface of the distal articles; their stimulation by vibrations of the substratum leads to the questing response.

The *palpal organ*, situated at the tip of the palps, is a chemoreceptor with a high threshold of stimulation. Its function lies in the attachment response rather than in orientation.

Temperature sensilla and tactile bristles are also present on the other legs and on the palps.

The reactions to light are due to a dermal sense.

4. *Attachment*

By offering warm or cold membranes of various types as sites for attachment, temperature, smell and a factor of the fleece which is perceived as a contact stimulus by the palpal organ, were found to provide the necessary stimulation for inducing attachment. Most ticks still attach to the natural host if the forelegs or the palps are amputated; none attach if both are lacking.

5. *Orientation mechanisms*

A questing response to light or vibration is often followed by movement. This mechanism (orthokinesis) is also very important in the humidity behaviour of the desiccated tick. Random changes in the direction of locomotion (klinokinesis) rarely occur. The usual response to favourable or unfavourable stimuli (e.g. temperature, smell and humidity) is directed and involves successive comparisons of intensity by the sensilla borne on the forelegs (klinotaxis). There is no tropotactic component, for ticks lacking one foreleg can still locate such stimuli accurately.

6. *Orienting responses in the natural environment*

An important physical feature of the rough moorland grazings which form the main habitat of the sheep tick is the steep humidity gradient within the vegetation layer. Near the roots, where the tick remains quiescent for long periods, the atmosphere is permanently moist; at the tips of the vegetation, where the tick comes to rest during the 'active' period, humidities are variable but generally lower. All the sensory perceptions are of value in promoting survival or host-finding. (*a*) First, certain reactions guide the tick to a situation—the vegetation tips—favourable for encountering the host. Of particular value in this respect is the upward-turning response summarized under § 2 (*a*). The tendency to remain near the tips is assisted at first by the humidity response, for ticks walking on the stem lattice avoid the high humidity near the roots. After an unsuccessful period of waiting the tick becomes desiccated and the kinesis comes into play: walking downwards the tick comes to rest at the roots, takes up water from the damp atmosphere, and is then prepared for a further period of activity at the tips. (*b*) Secondly, responses to tactile stimuli (unusual movements of the vegetation) and to light (shading) provide warning of

the imminent approach of the host. (*c*) Thirdly, orientation to the warmth and scent of the skin take place when the host is very near: the tick then catches hold and climbs on.

After dropping from the host the movements of the engorged tick down into the deeper layers of the vegetation are guided by the avoidance of directed illumination and to some extent by sensations of contact.

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